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Florencia

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List of abbreviations

5-hydroxytryptamine	5-HT
Acholecystokinin	CKK
Adverse Event	AE(s)
Area above the curve	AAC
Area under the curve	AUC
Autonomic nervous system	ANS
Body mass index	BMI
Central nervous system	CNS
Cyclic guanylyl monophosphate	cGMP
Dyspepsia symptom score index	DSSI
Enteric nervous system	ENS
Epigastric pain syndrome	EPS
European Medicines Agency	EMA
Functional dyspepsia	FD
Functional gastrointestinal disorders	FGID
Gastric accommodation	GA
Gastric emptying	GE
Gastroesophageal reflux disease	GERD
Gastrointestinal	GI
Glucagon-like peptide	GLP-1
Good Clinical Practice	GCP
H. Pylori	HP
Half emptying time	T ½
Healthy volunteer(s)	HV(s)
High resolution manometry	HRM
International Conference on Harmonization	ICH
Intragastric pressure	IGP
Irritable bowel syndrome	IBS
Leuven Postprandial Distress Scale	LPDS
Low esophageal sphincter	LES
Migrating motor complex	MMC
Minimal distending pressure	MDP
Minimum Clinically Important Difference	MCID
Nepean Dyspepsia Questionnaire	NDI
Nitric oxide	NO
Non-adrenergic non-cholinergic	NANC
Non-steroidal anti-inflammatory drugs	NSAIDs
Not significant	NS
Overall Symptom Severity	OSS
Overall Treatment Evaluation	OTE
Patient reported outcome	PRO
Phosphodiesterase-5	PDE5

Postprandial distress syndrome	PDS
Proton pump inhibitor(s)	PPI(s)
Quality of life	QoL
Selective serotonin reuptake inhibitor	SSRI(s)
Serotonin	5-HT
Short form-Nepean Dyspepsia Questionnaire	SF-NDI
Simptom index	SI
Standard error of mean	SEM
The joint hypermobility syndrome	JHS
Three times a day (ter in die)	t.i.d.
Transient receptor potential vanilloid type 1	TRPV1
Upper esophageal sphincter	UES
USA Food and Drug Administration	FDA
Vasoactive intestinal peptide	VIP
Visceral Pain neuro-matrix	VPN

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Chapter 1

General introduction, hypotheses and aims

Fragments of this chapter are published in a slightly different form in **Carbone F**, Tack J. Gastroduodenal mechanisms underlying functional gastric disorders. Dig Dis. 2014;32(3):222-9.

1.1. The stomach

The gastrointestinal (GI) tract includes the mouth, oesophagus, stomach, small intestine and large intestine that are connected to the vascular, lymphatic and nervous systems to facilitate the digestion and absorption of nutrients (1).

From proximal to distal, the stomach can be divided into four parts: the cardia, the fundus, the corpus and the antrum. It is limited by two sphincters, one at the end of the esophagus, the lower esophageal sphincter (LES), and one at the transition to the duodenum, the pylorus (Figure 1).

The gastric wall of the stomach is composed of four layers: serosa, muscularis, submucosa and mucosa layers. The serosa layer is formed by a thin layer of loose connective tissue, called the peritoneum, which is attached to the muscular layer. The muscular layers include outer longitudinal and inner circular smooth muscles that determine gastric motility. The submucosa contains blood and lymphatic vessels, and the nerve plexuses that form part of the autonomic nervous system contains sympathetic and parasympathetic fibres. Finally, the mucosa layer plays an important role in the first defense line against pathogens. It is lined with gastric glands that open into the gastric pits and greatly increase the mucosal surface area and include four major types of secretory epithelial cells cover the surface of the stomach. At the inner mucosa layer, cells secrete alkaline (HCO3-) mucosa that protects the epithelium against stress and acid. At the outer mucosa layer, parietal cells secrete hydrochloric acid (HCl+), chief cells secrete pepsin, a proteolytic enzyme, and G cells secrete the hormone gastrin (2) (Figure 1).

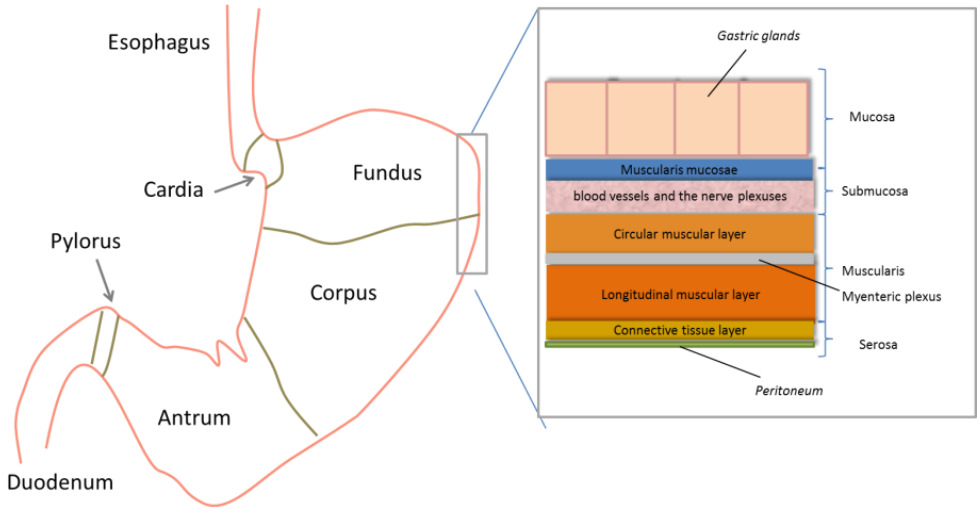

Figure 1: The anatomy of the stomach

1.2. Brain-gut interaction

The gastrointestinal system has its own extensive neural network that comprises the enteric nervous system (ENS) that supplies the intrinsic innervation, and the autonomic nervous system (ANS) that supplies extrinsic information. The ENS is present throughout the entire GI tract, including the myenteric plexus with neurons involved in the control of the GI motility, and the submucous plexus with neurons involved in the control of secretion, absorption and blood flow (2, 3).

The ENS contains three functional categories of neurons: sensory neurons, interneurons and motor neurons (4). The ANS innervation of the gastrointestinal tract comprises the parasympathetic (vagal

and sacral nerves) and the sympathetic innervation. In the proximal gastrointestinal tract, a major role is played by the vagus nerve. The intramural spinal and vagal sensory afferent neurons are sensitive to chemical, mechanical and thermal stimuli respectively by means of chemoreceptors, mechanoreceptors and thermoreceptors. These vagal afferent neurons send information to secondary neurons in the solitary tract nucleus in the brain that ascend to the thalamus, and then to a third-order of neurons that project to the sensory cortex. By means of these afferent signals, pathways mediating arousal, homeostatic and emotional behavior are involved that are necessary, among others, to regulate the digestive process and eating behavior, such as the feeling of satiety leading eventually to the termination of the meal (5). After processing of information from intrinsic afferents and vagal efferent neurons, ENS interneurons drive to an integrated output of motor neurons that control motility and secretion by acting directly on a variety of effector cells such as smooth muscle cells, secreting or absorbing epithelial cells and enteric endocrine cells (4, 5). To do this, activity of motor neurons can be stimulated or suppressed by neurotransmitters released at the multiple synaptic connections from interneurons. Stimulatory output from the ENS is mainly driven by the excitatory neurotransmitter acetylcholine (Ach) acting at nicotinic and muscarinic receptors to regulate secretory functions and smooth muscle contractility (6, 7). Smooth muscle relaxation is induced by means of inhibitory neurotransmitters such as nitric oxide (NO) and vasoactive intestinal peptide (VIP) released from non-adrenergic non-cholinergic (NANC) motor neurons (8-11). By means of this complex network of nerves, the ENS is capable of mediating reflex activity without the input of the brain stem and/or spinal cord, this model is called the heuristic model (Figure 2)(4).

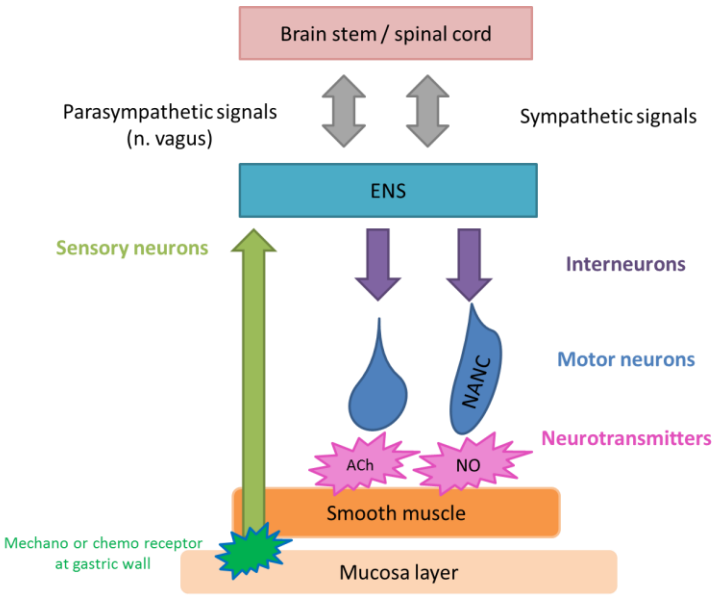

Figure 2: The gut-brain interaction

1.3. The digestion

During the digestive process, the meal in the mouth is chewed and mixed with saliva to prepare the bolus to be swallowed. The saliva also initiates the enzymatic digestion of starch (amylase) and lipids (lipase) and provides the first antimicrobial actions (12). During swallowing, the coordinated motor pattern, called primary peristalsis, is initiated in the esophagus. The bolus rapidly progresses through the pharynx and relaxed upper esophageal sphincter (UES) into the esophageal body, and by means of

progressive circular contractions, it proceeds distally along the esophageal body to propel the bolus through the relaxed LES through the cardia into the proximal stomach (13).

The stimulation of mechanoreceptors in the mouth and esophagus induces vago-vagal reflexes that cause a brief relaxation of the proximal part of the stomach, called receptive relaxation, in order to prepare the stomach to receive the food bolus (14-16).

The gastric wall contains two types of mechanoreceptors, one type which is arranged in a parallel fashion and reacts to the elongation of the stomach wall, and; another one arranged in series that reacts to increased tension of the stomach wall (17, 18). During the filling of the stomach, mechanoreceptors induce an adaptive relaxation of the fundus through the nerve vagus, in order to enhance its reservoir function. This reflex is called gastric accommodation (GA) (19, 20). This allows the proximal stomach stores the food before it is distributed to the distal part of the stomach.

During the GA, myenteric NANC nerves in the gastric wall are activated and generate NO. NO diffuses into the gastric smooth muscles activating the cGMP pathway that leads to relaxation of the muscles (21, 22). Besides the neurovagal NO-cGMP pathway, the GA reflex is related to a range of other complex regulatory pathways, not yet fully elucidated, where modest alterations, e.g. in luminal content in the gastrointestinal tract, can lead to significant changes in gastric motility. Vasoactive intestinal polypeptide (VIP) also acts as an inhibitory neurotransmitter in the proximal stomach (10, 11). VIP is also produced by the NANC neurons and diffuses to visceral smooth muscles cells where it initiates gastric relaxation through the cAMP pathway.

Serotonin (5-HT) has also been implicated in the control of the GA reflex, perhaps at the interneuron level (23, 24). Among others, studies using alosetron, a 5-HT_3 receptor antagonist (25), cisapride, a 5-HT_4 receptor agonist and 5-HT_3 receptor antagonist (26), paroxetine, a selective serotonin re-uptake inhibitor (27) and buspirone, a 5-HT_{1A} receptor agonist (28, 29), have shown the potential of 5-HT receptor ligands to enhance GA, although not all results are consistent (30, 31). In addition, endocannabinoid pathways have also been implicated in the control of GA (32).

Finally, the presence of nutrients in the small intestine also induces relaxation of the proximal stomach, as part of a negative feedback loop (33). The time of termination of the meal or the size of the meal is in part determined by satiety signals mediated through activation of mechanoreceptors and chemoreceptors sensing the meal volume and the presence of nutrients in the stomach and duodenum (20, 34).

Distention of mechanoreceptors in the stomach wall due to the increase in gastric content, for instance during balloon distention, leads to the activation of the visceral pain neuro-matrix (VPN), which is also associated with pain and discomfort generation (20). This neuro-signaling could enhance a satiety effect that leads to the sensation of satiation and fullness during the meal (20, 35). Tack *et al.* observed in healthy volunteers, that impaired GA, induced pharmacologically through the inhibition of NO-synthase by means of L-NMMA, leads to early termination of the meal due to an earlier sensation of satiation (36).

After the meal, tonic contractions of the proximal stomach cause a redistribution of the gastric content to the distal stomach (20). In the antrum, the food will be ground and crushed by means of peristaltic waves (propulsion and retropulsion), and mixed with gastric juices to homogenize the food into a mix of smaller particles (less than 1 to 2 mm) called the chyme (16, 37, 38).

The emptying of a solid meal is divided in two phases. During the lag phase, peristaltic waves originate from the stomach corpus to the antrum forcing the chyme towards the pylorus which remains closed,

thereby contributing to grinding up of food particles. This phase is followed by a phase of constant emptying of the nutrients into the duodenum (39).
In the small intestine, the chyme is further dissolved with help of juices from the pancreas, liver, and intestine as the digested nutrients are absorbed through the intestinal mucosa (16, 37). Moreover, nutrient and caloric sensing in the small intestine drives the release of hormones and other signaling molecules into the circulation such as cholecystokinin (CCK), glucagon-like peptide (GLP-1) and Peptide YY (PYY), which further enhance the satiation signals that lead to the gratifying and rewarding sensation of fullness after the meal (20, 40). Furthermore, peristalsis ensures further migration of the waste products a through the jejunum and ileum to arrive at the colon for excretion through the anal sphincter.

1.4. Functional dyspepsia

Dyspepsia refers to a heterogeneous group of symptoms present in the epigastric region such as early satiation, epigastric pain, postprandial fullness, nausea or upper abdominal bloating (41, 42). The Rome consensus subdivided functional gastrointestinal disorders (FGID) into six major domains corresponding to the area of the gastrointestinal tract where symptoms are thought to originate. Functional dyspepsia (FD) one of the most common FGID and is defined by the Rome consensus as the presence of one or more symptoms thought to originate from the duodenum, occurring for more than 6 months and that cannot to be explained by the presence of any organic or metabolic disease (41, 42). Although the majority of dyspeptic patients do not seek medical attention, the prevalence of dyspepsia in the population is very high, at 15% to 20%, with an incidence of 1% per year (41-43). Although this disease is not lethal, the symptomatic impact is considerable and quality of life of these patients is significantly impaired. High absenteeism, impaired productivity and high medical costs are associated with FD, but no impact on mortality has been found (43-45).
Based mainly on expert opinion, the Rome consensus subdivided FD into two subgroups: epigastric pain syndrome (EPS), characterized by symptoms of epigastric pain and/or epigastric burning, and; postprandial distress syndrome (PDS) characterized by postprandial fullness and/or early satiation (41, 42). It was proposed that this subdivision would serve as a guide for the diagnostic and therapeutic approach to FD patients (41, 42, 46). In the general population, a good separation has been observed between EPS and PDS (47-51). However, in clinical practice a great overlap, in some cases up to 50 %, has been established, therefore limiting the value of these criteria (52-58).

1.4.1. Pathophysiology of FD

Despite extensive research, the underlying pathophysiology of FD symptoms remains unclear. The greatest difficulty is the heterogeneity of the symptom pattern in FD patients. A possible dysregulation on the gut-brain axis is considered to play a key role in this disorder (41, 59, 60). However, it is not yet known what specifically triggers symptoms, and this is likely to be part of a complex relationship between brain and gut triggered by psychosocial co-morbidities, as well as gastric motor and sensory dysfunction (22, 61-63). Moreover, impaired duodenal mucosal integrity, low-grade immune activation and changes in gastroduodenal chemosensitivity have also been implicated. Most recently, there has been an increasing awareness of the co-existence of the joint hypermobility syndrome (JHS) with functional GI disorders, with a particularly strong association with dyspeptic symptoms (64-66).

a. Impaired gastric emptying

Gastroparesis is defined as the presence of symptoms (nausea and vomiting, early satiety, postprandial fullness, bloating, and upper abdominal pain) and delayed gastric emptying in the absence of mechanical obstruction (67-69). Delayed gastric emptying can be diagnosed by different tests such as scintigraphy gastric emptying test (the current gold standard) and the breath test (67, 70), and can be associated with systemic disorders (e.g. diabetes, neurological disorders) or can be idiopathic.

I. Idiopathic gastroparesis

In idiopathic gastroparesis, it has been hypothesized that a prior viral or bacterial infection may trigger disordered gastric neuromuscular control mechanisms, leading to gastric emptying malfunction (68, 69). However, the pathophysiology of idiopathic gastroparesis is poorly understood, and also there is a great overlap between the symptom manifestations of idiopathic gastroparesis and FD, specifically for PDS (68, 71). Both are upper gut functional disorders with very similar symptom classification and treatment management. This leads to a matter of controversy between patients, physicians and researchers on what constitutes PDS and what constitutes idiopathic gastroparesis. As nausea and vomiting are considered the cardinal symptoms of gastroparesis; the Rome III criteria attempted to distinguish FD from gastroparesis by discriminating nausea and vomiting as a category of functional GI disorder separate from FD (68, 71, 72). In support of this approach, Sarnelli *et al.* had shown that delayed gastric emptying in FD patients is mostly associated with nausea, vomiting and also postprandial fullness (73). However, delayed gastric emptying can be identified in about 30% of FD patients and the association with a pre-defined symptom pattern is relatively poor (74).

II. Diabetic gastroparesis

Gastroparesis is more prevalent in patients with diabetes. Longstanding diabetes mellitus could affect the function of every organ of the body and GI complications are very common in this group of patients (75). The ten year incidence estimation of diabetic gastroparesis is 5.2% in type-1 diabetes and 1% in type-2 diabetes (69, 76). The pathophysiological mechanisms associated with abnormal gastric motor functions in diabetes mellitus patients includes macro- and micro-vascular complications, poor glycemic control, extrinsic and intrinsic enteric neuropathy, abnormalities of interstitial cells of Cajal, impaired nitrergic innervation, smooth muscle fibrosis and impaired neurohormonal factors (67, 69, 75). Finally, other causes contributing to gastroparesis might be the use of medications with motor-inhibitory effects such as including anticholinergics, narcotics, tricyclic antidepressants, and calcium channel blockers, but especially glucagon-like-1-peptide analogues in diabetes (67, 77).

III. Other causes

Gastroparesis is also occurring after a surgery or trauma that impairs integrity of the vagus nerve that regulates fundic relaxations and antral contractions (67, 69, 77).

b. Impaired gastric accommodation

In FD, impaired gastric accommodation to a meal is considered to be a major pathophysiological mechanism. It has been observed in up to 40% of FD patients (22). It has also been suggested that impairment of GA contributes to the generation of symptoms in FD patients such as nausea, bloating, early satiety and weight loss (40).

Bisschops *et al.* demonstrated that most FD patients experience symptoms that are triggered or aggravated by a meal (78). In this study, 30% of the patients had impaired GA. Moreover, the timing of symptoms relative to the meal differed. Most clearly triggered by the meal were postprandial fullness and bloating, reaching maximum intensity in the first postprandial half hour, while epigastric pain and also epigastric burning or nausea had a much later postprandial peak. In FD, the generation of the symptoms could be due to a combination of antrum and fundic dysfunction. Salet *et al.* reported a different response to gastric distention in healthy volunteers and FD patients (40). In an imaging study, Troncon *et al*. showed that nutrient distribution to the proximal stomach was decreased in FD compared to healthy subjects (79). Furthermore, Caldarella *et al.* reported an increased sensitivity to distention of both the fundus and antrum of FD patients compared to healthy subjects (80). It has been hypothesized that impaired GA could lead to antral overload and therefore trigger symptoms both from the fundus and the antrum in FD patients.

c. Visceral hypersensitivity

It has been previously observed that a subgroup of FD patients display visceral hypersensitivity (18, 81, 82). Gastric sensitivity can be determined by means of the gastric barostat (explained in detail in Section 1.5.1). Hypersensitivity to gastric distention has been defined as a decrease of the first perception threshold and the discomfort and pain threshold (18). A study of Tack *et al.* studied the sensitivity to gastric distention in healthy volunteers and FD patients (61). The results showed that hypersensitivity for gastric distention is present in a subset of patients when comparing the sensation thresholds and that hypersensitivity to distention is associated with symptoms of postprandial pain, belching and weight loss. Another study with the gastric barostat showed that in FD patients with a normal gastric emptying and normal GA, administration of the stomach relaxatory drugs sumatriptan and clonidine, decreased the severity of dyspeptic symptoms (83). As the relaxatory effect was accompanied by a decrease in sensitivity to gastric distention, these observations are in agreement with the possible involvement of tension-type mechanoreceptors in mediating dyspeptic symptoms.

A great number of FD patients report that their symptoms were triggered or aggravated after the meal (78). Therefore, it has been suggested that postprandial hypersensitivity might be most relevant to the generation of FD symptoms. However, a number of variables that might affect the gastric sensitivity after a meal should be taken into account such as GA, gastric acid secretion, gastro-duodenal hormone secretion, and the nutrient composition of the meal (21, 61, 84-87). In a study by Farré *et al.*, postprandial gastric sensitivity to distention correlated to the cumulative meal-related symptom score for painful sensations and for non-painful sensations, such as fullness, bloating, and belching (87). Moreover, it was suggested that in FD, interaction between the sensitivity of mechanoreceptors and the GA reflex to a meal might determine postprandial sensitivity to gastric distention and hence symptom generation.

An alternative to explore the visceral sensitivity independent of the mechanoreceptors in FD patients is by means of chemical stimulation using stimuli such as capsaicin and intragastric or intraduodenal

acid infusion. Capsaicin, the natural compound of chili pepper, binds to the transient receptor potential vanilloid type 1 (TRPV1) channel and converts thermal and chemical stimuli into painful sensations or discomfort (88, 89). Moreover, it has been previously observed that the TRPV1 channel could also be involved in gastric motility regulation. Studies with capsaicin in FD patients have showed an increase in hypersensitivity and dyspeptic upper gut symptoms (82, 86, 90, 91). However, the known hypersensitivity in the subjects was not a predictor for symptoms and their intensity (86). Furthermore, Oshima *et al*. studied the effect of acid and water infusion in the stomach of FD patients and healthy subjects (92), showing that FD patients were more sensitive to the acid infusion compared with the healthy volunteers. Patients reported an increase in the quantity and severity of dyspeptic symptoms with acid when compared to water infusion. Studies in FD patients have reported that duodenal clearance of exogenously infused acid was reduced and that patients are hypersensitive to exogenous acid infusion (93). Moreover, the most prominently induced dyspeptic-related symptom in FD patients was nausea; however, this was not the case in healthy volunteers (93, 94). A study in FD patients with prominent nausea showed that patients had an increased endogenous acid exposure during daytime with reduced duodenal clearance (85). It was also observed that the severity of the symptoms was related with the increased endogenous acid exposure. However, infusion of exogenous acid in the duodenum did not aggravate the symptoms. Prolonged duodenal acid infusion in healthy subject induces dyspeptic symptoms such discomfort, bloating, nausea and epigastric burning (95). Finally, it has also been shown that duodenal acid infusion of exogenous acid in the duodenum decreases the threshold for discomfort to gastric balloon distention and inhibits GA to a meal (84).

1.4.2. Management of FD

a. Diagnosis

After careful medical history taking and physical examination, in dyspeptic patients some routine blood tests are often added to exclude a possible metabolic disease. In addition, making a diagnosis of functional dyspepsia also requires an upper gastrointestinal endoscopy to exclude the presence of organic diseases such as esophagitis, gastric ulcer, malignancy and GI infections such as giardiasis. Management of FD firstly involves education and explanation to the patient of the benign but persistent nature of the disorder. Due to lack of curative therapies, the current management approach to FD focuses on the reduction of symptoms (96, 97).

b. Eradication of H. Pylori

The presence of *H. Pylori* can be determined by histological studies or by non-invasive diagnostic tests such as the ^{13}C-urea breath test, and, when positive, its eradication is the recommended first-line treatment (98). Lately, the Kyoto consensus has stated that H. pylori infection can be implicated in the pathogenesis of FD if symptoms disappear after long term successful eradication of *H. Pylori*. This is referred to as for *H. pylori* -associated dyspepsia; if dyspeptic symptoms persist these patients are referred to as having FD (41, 98) (Figure 3). *H. Pylory* eradication is associated with a small but significant benefit over no eradication as 8-14 is the estimated number needed to treat (98). However, it is difficult to predict whether a patient with dyspeptic symptoms will respond to eradication therapy or not (98).

c. Adaptation of lifestyle and diet

As in a large subset of FD patients, especially PDS patients, meals play an important role in triggering or worsening symptoms (43, 78, 99, 100), adapting the patient's daily diet is often used to try and improve symptoms; although this aspect has been poorly studied. Patients are instructed to avoid fatty, high calorie foods and to consume smaller-sized meals in order to avoid triggering symptoms such as fullness, bloating and epigastric burning or heartburn (96, 97, 100-102). However, only one study has been able to show a correlation of the ingested amount of fat with bloating, and the ingested amount of calories with postprandial fullness (103).

d. Proton pump inhibitors

Based on the symptom pattern, the first-line pharmacotherapy choices include antisecretory drugs, especially proton pump inhibitors (PPIs). These drugs are more likely to be effective in EPS symptoms. The use of prokinetic agents seem to be more effective in FD patients with PDS symptoms, although the available evidence there is limited (96) (Figure 3). Studies have shown that the use of PPIs such as omeprazole, lansoprazole and pantopranzole in FD patients with some degree of reflux or heartburn, or in the EPS subgroup, have been more likely to respond to the therapy, although this improvement has not always been significant when compared to a placebo (104-107). The effectiveness of PPI treatment in FD is modest with a therapeutic gain of approximately 7–10% (97). Moreover, a systematic review of eight randomized clinical trials with omeprazole and lansoprazole in FD showed a number needed to treat of 9 (108). The therapeutic effect of PPIs might be explained by control of a GERD component and to the beneficial effect of these drugs on acid induced inflammation or acid-induced hypersensitivity in these patients (97, 108).

e. Prokinetics

PDS or dysmotility-like dyspepsia has been treated with prokinetics in older studies showing promising results for targeting gastric motor functions. Cisapride (a 5‑HT4 receptor agonist) and domperidone (a dopamine‑2 receptor antagonist) known to stimulate gastric motility have shown improvement in FD (109-111). A meta-analysis of studies with domperidone and cisapride showed improvement over placebo with a relative risk reduction of 33% and a number needed to treat of 6 (112). Cisapride enhances gastric emptying and intestinal transit, increases postprandial gastric volume and increases volume tolerance in a drinking test (26, 110). Besides its prokinetic function, domperidone also has antiemetic activity as a result of blockade of dopamine receptors in the central chemoreceptor trigger zone (111, 113, 114). However, cisapride has been withdrawn in many countries because of cardiac safety concerns (arrhythmia though the QT-prolongation thought to be a blockade of hERG voltage-gated potassium channels), and domperidone is not widely available and has also been shown to prolong QT intervals (110, 115). Among 5‑HT4 agonists, mosapride has shown small symptomatic benefit (116) and short-and long-term studies with tegaserod showed a modest benefit of modest benefit with satisfactory relief of 4.6% over placebo (97), although the drug has mainly been implemented as a treatment for IBS, and the drug has been withdrawn in most parts of the world for cardiovascular safety issues (117-120). Levosulpiride, a D2-dopamine receptor antagonist and a serotonin 5HT4 receptor agonist, has shown efficacy on relieving FD symptoms by altering gastrointestinal motility and sensitivity. Comparative studies showed it to be at least as effective as

cisapride and domperidone (121-124). Erythromycin is a motilin agonist that has shown therapeutic efficacy in gastroparesis (125-127). However, the symptom benefit is limited and long-term antibiotic use is an unattractive option (128). Itopride, a dopamine-2 receptor antagonist and cholinesterase inhibitor, showed promising results in a phase II study, but the results from phase III studies were disappointng (129-132). Acotiamide, a novel first-in-class agent, enhances acetylcholine release via antagonism of M1 and M2 muscarinic receptors and also functions as a cholinesterase inhibitor. Phase II studies with acotiamide (100 mg) compared to placebo in FD patients showed a consistent improvement rate of approximately 10% using the OTE questionnaire as a primary endpoint. Postprandial fullness was the most responsive symptom, with a high elimination rate in the acotiamide group compared to the placebo group (133-135). Phase III clinical trials conducted in Japan have demonstrated significant improvement of FD PDS patients with acotiamide in comparison with a placebo group. The safety profile was demonstrated to be excellent and the FD symptoms were improved or resolved in up to 38% of the patients and that the number needed to treat of 6 for overall treatment efficacy (136).

f. Antidepressants- antipsychotics

For non-responders with a bothersome impact on daily functioning, it is suggested that treatment options may involve combinations of PPI and/or prokinetics, antidepressants or referral for cognitive behavioral therapy, relaxation therapy or hypnotherapy (96). Furthermore, the rationale to use psychoactive agents is based on their potential effect on co-morbid psychiatric and psychological conditions, central analgesic actions on visceral pain and local pharmacological actions on upper gastrointestinal motility (27, 28, 96) (Figure 3). Hojo *et al.* confirmed the efficacy of antidepressants with a relative risk reduction of symptoms of 0.55 in a meta-analysis of mostly tricyclic antidepressants (137). Nevertheless, it has to be taken into account that many of these studies did not exclude concomitant depression (137).

Buspirone, 5-HT1A agonist and anxiolytic agent, seems a promising drug that enhances GA and has potential to improve symptoms in FD patients (28, 138). Mirtazapine is an antidepressant that blocks the re-uptake of noradrenaline and has antagonistic effects on central noradrenergic and serotonergic (specifically 5-HT3) pathways. It is known to stimulate appetite and weight gain in depressed patients, enhances gastric emptying rate and recently an increased nutrient tolerance was reported in FD patients (139-144). Finally, amitriptyline appears to improve dyspeptic symptoms, especially pain and nausea in FD patients (145-147).

Nevertheless, the efficacy of these therapies is limited and the development of novel options is hampered by difficulty to achieve optimized patient selection, overlap between FD subgroups and other functional GI disorders and the use of inappropriate endpoints or endpoint questionnaires (148, 149). Patient reported outcome (PRO) questionnaires provide information on specific health concepts directly from the subjects without interpretation of the patient's response by a physician or others, and are used to evaluate treatment responsiveness in patients (148, 150). To date, no validated PRO is available to evaluate symptom severity in FD in line with Rome III and the US Food and Drug Administration (FDA) guidelines. In addition, the large observed placebo response in clinical trials (30-40%), the heterogeneity of the condition, and the difficulty in establishing the underlying pathophysiology in individual patients can also largely affect the development of efficacious therapies (41).

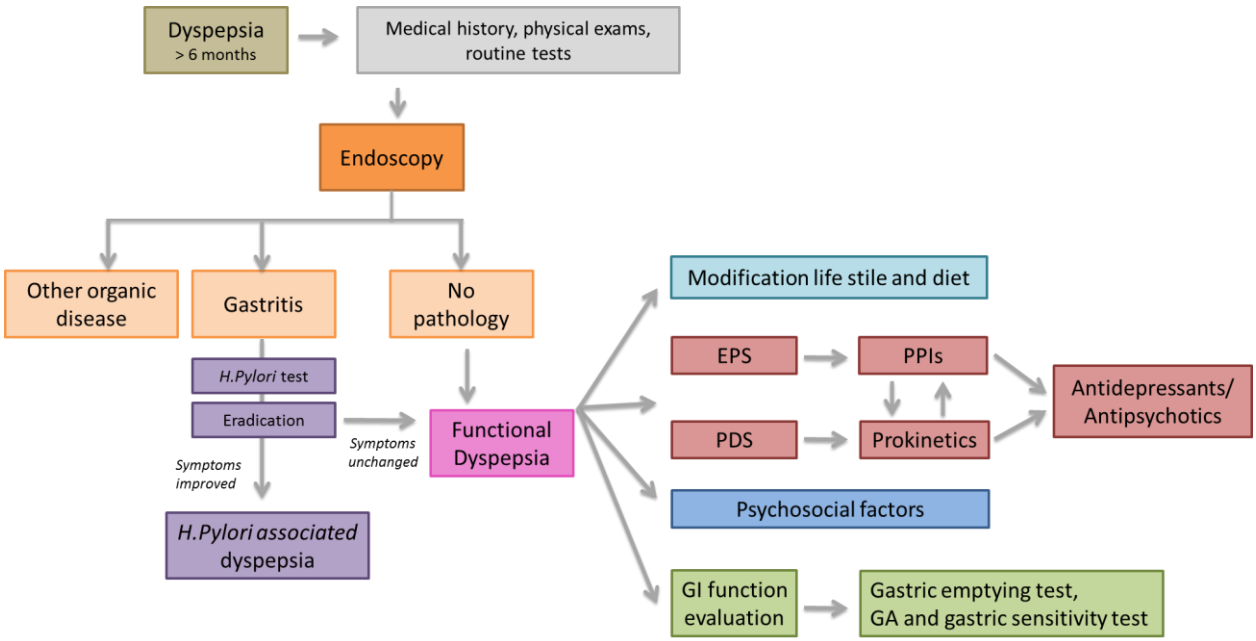

Figure 3: Clinical algorithm for the management of FD

1.4.3. Assessment of gastric accommodation and visceral sensitivity

a. The gastric barostat: the gold standard

The gastric barostat is considered the gold standard to study the gastric sensitivity to distension as well as changes in gastric tone in fasted and fed state. The gastric barostat consists of a polyethylene bag with a maximum capacity of 1000-1200 milliliters attached to a double-lumen polyvinyl tube. The polyvinyl tube is connected to a programmable barostat device that allows isobaric or isovolumetric expansion of the barostat balloon and measures the air volume in the balloon maintained at a constant pressure. The pressure in the gastric balloon is first equilibrated to the intra-abdominal pressure of the subject, termed the minimal distending pressure (MDP) and using balloon pressures above MDP implies the position of the balloon unwrapped and in contact with the gastric wall (151). By means of step-wise isobaric distensions of the barostat in the stomach, gastric compliance and visceral perception can be assessed. During the ingestion of a liquid nutrient meal, the proximal stomach relaxes and GA can be measured as an increase in the barostat volume (59, 151-154).

This procedure is invasive, uncomfortable, and patients perceive it as difficult to tolerate and stressful. The barostat bag allows measuring the fundic relaxation, but information about antral responses is missed. There are many technical pitfalls leading to difficult interpretation of results such as the assessment of a negative accommodation, observed in 10% of interpretable studies, or the presence of an abnormal motility index suggestive of unsuppressed postprandial phasic contractility, observed in 15% of the studies. Additionally, 1.7% of the patients are not able to tolerate the bag insertion or request to abort the procedure at an early stage (155). When calculating mechanical factors such as gastric distension, the entire surface of the balloon must be in contact with the organ wall and this can only be assumed by the distention level (156, 157). Moreover, the presence of the barostat bag may alter the intragastric distribution of a meal and it may exaggerate the relaxation of the proximal and distal stomach due to the direct distension stimulus of the balloon on the stomach wall (40, 158, 159).

b. New alternative to the barostat: the high resolution manometry

High resolution manometry (HRM) uses a catheter composed of multiple closely spaced pressure sensors (36 sensors 1 cm apart) that can be used to measure intraluminal pressure changes of the GI tract. The pressure data is transformed into pressure waveforms which are color-coded as cool colors (blue and green) for lower pressures and warm colors (red and yellow) for high pressures. The technique is widely available and uses an identical catheter as esophageal manometry and therefore, has the potential to gain similar acceptance and feasibility level as an esophageal manometry.

To study GA, a HRM probe is positioned through the nose into the stomach with the sensors distributed from the proximal to the distal stomach. Therefore, the HRM permits the simultaneous recording of pressures in different portions of the stomach, but also in the distal esophagus and proximal duodenum. To measure GA, the IGP is measured as the average pressure of the first 5 pressure channels that are clearly positioned below the low esophageal sphincter (LES) or the pressure area influenced by the LES (approximately 3-8 cm under the LES) (160).

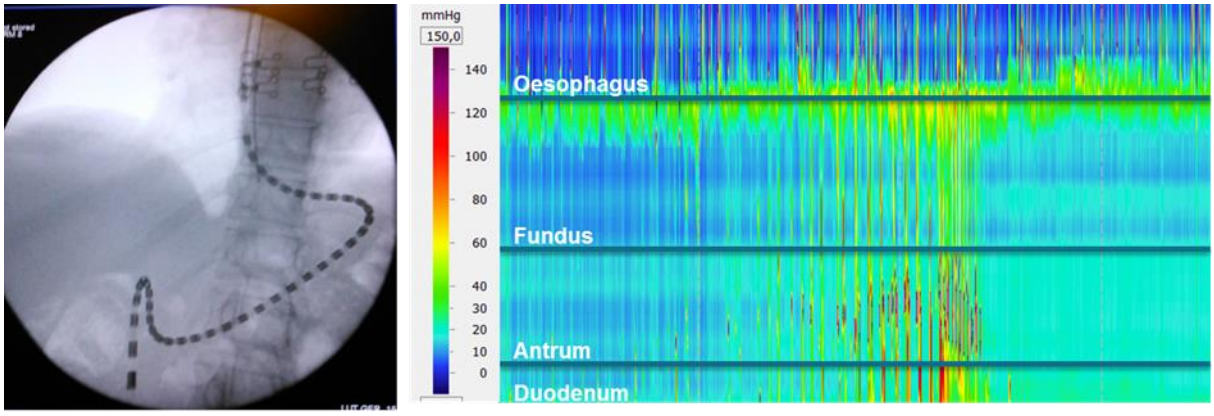

Figure 4: The left hand panel depicts a fluoroscopic image of the position of the HRM probe in the stomach. The right hand panel depicts a color plot of the intragastric pressure measurement over time. High pressures are shown in red and purple colors, and the low pressures are shown as green and blue colors. The location of the high pressure zone of the LES (low esophageal sphincter), as well as the representation of the fundus, antrum and duodenum are indicated. The latter two are identified by the presence of pressure rises resulting from antral and duodenal contractions respectively. During the intragastric infusion of a meal, the proximal stomach relaxes (IGP drop) and this can be observed as a darker blue color in the color plot.

During a meal, the proximal stomach relaxes and this is assessed as a drop of pressure from baseline. The IGP measurement can be combined with the assessment of volume-nutrient tolerance by intragastrically infusing a liquid nutrient meal until the subject reports maximal satiation (160). This method follows the principle of the slow nutrient drinking test that provided an indirect measurement of GA by assessing the symptom of (early) satiety suggesting that the ingested quantity of a liquid nutrient meal could be used as a surrogate marker of (impaired) fundic relaxation (161-163).

The sensitivity of the IGP to detect abnormal gastric accommodation and satiation has been previously studied in an experimental pharmacologic set-up. NO is the main inhibitory neurotransmitter involved in the GA reflex. Using the gastric barostat, it was shown that the administration of the selective NO

synthase inhibitor, NG-monomethyl -L- Arginine (L-NMMA) inhibits GA and decreased liquid meal tolerance in the nutrient drink test (164, 165). Using HRM, similar results were observed. Pre-treatment with L-NMMA was shown to increase fasting muscle tone, to inhibit the meal-induced IGP drop and to induce early satiation in healthy subjects (160). A second study also presented similar results with both techniques. Rotondo *et al.* showed in a combined study with the barostat and IGP measurement that liraglutide, an analogue of the anorexigenic hormone GLP-1, inhibited gastric accommodation and simultaneously inhibited nutrient volume tolerance (166).

These small sets of studies were able to demonstrate that IGP measurement in combination with intra-gastric infusion until maximal satiation might be a good alternative to measure (impaired) gastric accommodation compared to the barostat. Nevertheless, this technique remains very novel and further validation and interpretation of the assessed parameters is needed.

1.5. Objectives of the research

Taking into account the gaps in the state of the art knowledge in FD, this thesis comprises different objectives. The first objective was to optimize the assessment of the symptom pattern, severity and correlation with underlying pathophysiology which are crucial for improving FD management. This was done firstly by exploring an approach to improve the subdivision and classification of FD into EPS and PDS subgroups by improving the characterization the FD symptom pattern and severity in the different subgroups, and by studying the link between FD symptoms and underlying pathophysiological mechanisms (Chapter 2). Secondly, we aimed at developing and validating a PRO questionnaire for PDS, based on the US FDA guidance for PROs for the PDS subgroup (Chapter 3). The choice to start with the PDS subgroup was driven by the larger proportion of PDS patients compared to EPS patients and by the availability of a large group of prokinetics that need to be studied in this patient group.

Understanding the underlying pathophysiological mechanisms in FD is necessary to target and develop new therapeutic options. Moreover, although impaired GA is an important pathophysiological mechanism, there is a lack of a suitable measurement technique to optimally study and completely understand GA. Therefore, the second objective was to further validate and apply IGP measurement to assess GA in response to food intake in healthy subjects and FD patients (Chapter 4). Also, to apply this measurement in an experimental set-up to explore in detail the physiological control mechanisms of IGP and the role of their possible alterations in generating dyspeptic symptoms (Chapter 4 and Chapter 5). Finally, we aimed at expanding the therapeutic abilities in FD by exploring alternative therapeutic pathways (Chapter 5 and Chapter6).

Chapter 2

Pathophysiology and validity of functional dyspepsia subgroups

2.1. Pathophysiological abnormalities in functional dyspepsia subgroups according to the Rome III criteria

In press in American journal of gastroenterology

2.1.1. Introduction

The current consensus definition for functional dyspepsia (FD) are the Rome III criteria which define the disorder as the presence of symptoms thought to originate in the gastroduodenal region, without any organic, systemic or metabolic disease that readily explain the complaints (41, 167). The prevalence of FD in the community according to this definition ranges between 5 and 11% (168). The Rome III criteria proposed to subdivide FD into two different diagnostic categories: postprandial distress syndrome (PDS, characterized by the presence of postprandial fullness and/or early satiety) and epigastric pain syndrome (EPS, characterized by the presence of epigastric pain and/or epigastric burning) (41, 167). The subdivision was based on the assumption that different pathophysiological mechanisms underlie different dyspeptic symptoms. However, it has been clearly demonstrated that major overlap exists between PDS and EPS symptoms in FD patients presenting to medical care (42, 169). A wide range of putative pathophysiological mechanisms have been investigated in FD (170). Disorders of gastrointestinal sensorimotor function such as impaired gastric accommodation, hypersensitivity to gastric distension, and delayed gastric emptying are highly prevalent in FD patients (16, 22, 61, 73, 81, 170-173) and have been proposed to contribute to dyspeptic symptom generation. Although the Rome III consensus panel proposed that different pathophysiological mechanisms are involved in PDS and EPS symptom generation (41), to date, this has not been extensively evaluated. Therefore, the aim of this manuscript was to evaluate differences in pathophysiological mechanisms and their association with symptom pattern and frequency in the Rome III FD subgroups.

2.1.2. Materials and Methods

Patients with functional dyspepsia

Consecutive FD patients were recruited from the outpatient clinic of the gastroenterology department at the University Hospitals Leuven. All patients completed a Rome III questionnaire assessing dyspeptic symptoms such as postprandial fullness, early satiety, nausea, bloating, epigastric pain and epigastric burning. The frequency of each symptom over the last three months was graded (0-5; 0=absent, 1=once a month or less, 2=two or three times a month, 3=once a week, 4=several times a week, 5=every day). The cumulative symptom score was calculated by adding up the score of postprandial fullness, early satiety, nausea, bloating, epigastric pain and epigastric burning. Based on these symptom scores, the patients were subdivided into two subgroups according to the Rome III consensus: (1) PDS, characterized by postprandial fullness and/or early satiety at least several times a week, and (2) EPS, characterized by epigastric pain and/or epigastric burning at least once a week. Moreover, a third group of patients who fulfilled the above mentioned Rome III criteria for both PDS and EPS was defined as the overlap group. We also calculated a PDS symptom severity score by calculating the sum of postprandial fullness and early satiety and an EPS symptom severity score by calculating the sum of epigastric pain and epigastric burning. The non-cardinal symptoms nausea and bloating were added to the analyses as individual symptoms because they occur very frequently in FD patients. Subjects were excluded if they failed to fill out the Rome III questionnaire adequately, if they

had abnormal findings on upper gastrointestinal endoscopy, or if they had a history of former upper digestive surgery, diabetes, predominant irritable bowel syndrome, celiac disease, inflammatory bowel disease or esophageal symptoms such as dysphagia or globus.
The current analysis was restricted to patients who underwent a barostat and/or a standardized gastric emptying test at our institution (see below). All drugs potentially affecting gastrointestinal motility or gastric acid secretion were discontinued at least 1 week prior to the barostat or gastric emptying study.

Barostat
After an overnight fast, the subjects were orally intubated with a double-lumen polyvinyl tube (Salem sump tube 14 Ch; Sherwood Medical, Petit Rechain, Belgium) with a finely folded adherent plastic bag (1200 mL capacity; 17 cm maximal diameter). The position of the bag in the gastric fundus was checked fluoroscopically.
After intubation, the subjects were asked to sit in a comfortable sitting position with the knees bent (80°) and the trunk upright in a specifically designed bed. The polyvinyl tube was then connected to a programmable barostat device (Synectics Visceral Stimulator, Stockholm, Sweden). To unfold the bag, it was stepwise inflated with a fixed volume of 300 mL air for 3 min and again deflated completely. After a 30 min adaptation period, the minimal distending pressure (MDP) was determined by increasing intrabag pressure by 1 mm Hg every minute until a constant volume above 30 mL was reached. After 15 min of rest, gastric sensitivity was tested with sequential isobaric distensions of the barostat balloon in stepwise increments of 2 mmHg starting from MDP, each lasting for 2 min, while the corresponding intraballoon volume was recorded. At the end of every distending step, the patients were asked to score their perception of upper abdominal sensations using a graphic rating scale that combined verbal descriptors on a scale graded 0-6 (61, 174). The end point of distensions was established when the subjects reported discomfort or pain (score 5 or 6). This pressure is referred to as the discomfort pressure. Gastric hypersensitivity to distension was determined to be present if the discomfort pressure <6.6 mm Hg above the MDP (61). After 15 min of recovery with the bag completely deflated, gastric accommodation was tested. The pressure level was set at MDP + 2 mm Hg for 90 min during which the subjects scored satiation (0-5) every 5 min. After the first 30 min, a liquid meal of 200 mL was administered (300 kcal; 12 g of proteins, 36.8 g of carbohydrates and 11.6 g of fat; Fortimel Energy, Nutricia, Amsterdam, the Netherlands). In all patients, the measurement continued for 60 min after the meal. Gastric accommodation was calculated by measuring the difference of the average volume at 30 min before (pre-prandial) and at 60 min after (postprandial) meal ingestion. It was defined as being ‘impaired’ if the increase in volume was less than 64 mL (22). A scheme of the gastric sensitivity and gastric accommodation testing is shown in Figure 1.

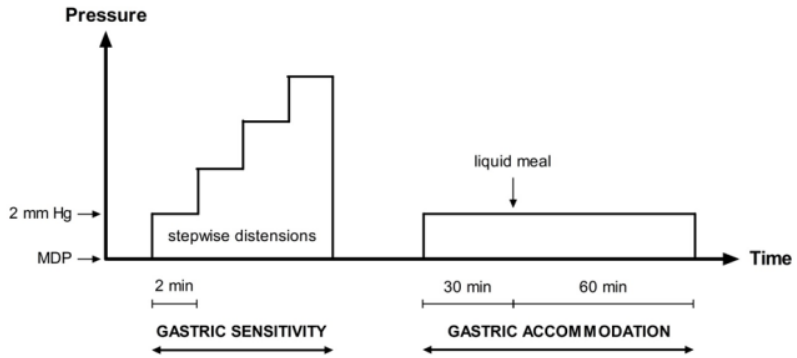

Figure 1. Schematic outline of the barostat study. MDP=minimal distending pressure

Gastric emptying

Gastric emptying for solids was determined using the previously validated [14C] octanoate breath test (175, 176). All tests were carried out in the morning after an overnight fast. Breath samples were taken before a standardized meal and at 15 min intervals for a period of 240 min after meal ingestion. The meal consisted of 60 g of white bread, one egg of which the yolk was doped with 74 kBq of [14C] octanoic acid sodium salt (PerkinElmer, Waltham, Massachusetts, USA) and 300 mL of water. All meals were consumed within a 10 min period. The total caloric value of the test meal was 250 kcal (14 g of proteins, 26 g of carbohydrates and 10 g of fat). 14CO2 was collected for each breath sample by blowing air through a pipette in a liquid scintillation vial containing 2 mmol of hyamine hydroxide and thymolphtalein as indicator. Blowing lasts until discoloration of the indicator, corresponding to the capture of 2 mmol CO2 by hyamine hydroxide. To detect the activity emitted by 14CO2, scintillation solution (Hionic Fluor, PerkinElmer, Waltham, Massachusetts, USA) was added and radioactivity was determined by liquid scintillation counting (Tri- Carb Liquid Scintillation Spectrometer, model 2910TR, PerkinElmer, Waltham, Massachusetts, USA). The breath test results of the liquid scintillation counting were expressed as disintegrations per minute. This was converted to percentage 14CO2 excreted per hour of the initial dose administered, assuming CO2 production to be 300 mmol m-2 of body surface per hour. Gastric half emptying time (t1/2) was calculated from the 14CO2 excretion curves as described previously (24, 25). Based on previous results in healthy volunteers, the threshold for delayed gastric emptying was defined as t1/2>109 min for a solid meal (73).

Statistical analysis

Data were analyzed using GraphPad Prism or SAS V.9.4; differences were considered significant when $P<0.05$. Differences between groups were analyzed using Chi-square tests for categorical variables and one way ANOVA or Kruskal- Wallis for continuous variables when appropriate, followed by post-hoc testing (Tukey or Dunns correction for multiple testing). Results are expressed as mean±SEM or median (IQR). Associations between pathophysiological mechanisms (continuous variables) on the one hand and cumulative symptom score (continuous variable) on the other hand was tested in each Rome III subgroup separately using Spearman's correlation due to the non-normal distribution of the data. Since PDS, EPS, and individual symptom scores (bloating, nausea) are ordinal data, mostly with very skewed distributions, they were divided in textiles or quartiles depending on their distribution, thereby converting them to ordinal variables with 3 or 4 levels.

Associations between pathophysiological mechanisms (continuous variables, log transformed to reduce the influence of outliers when appropriate) on the one hand and these ordinal symptom

variables on the other hand were analyzed in each Rome III subgroup separately using cumulative logit logistic regression analyses for ordinal outcome variables. Since these analyses imply performing many bivariate association tests, false discovery rate correction was applied to their resulting *P* values.

2.1.3. Results

Study population

A dataset of 560 FD patients (395 women, 165 men, age 41.8±0.7 years, body mass index (BMI) 22.4±0.2 kg m-2) fulfilling the Rome III diagnostic criteria was analyzed.

These patients were subdivided into three subgroups according to the Rome III consensus: 23% PDS (89 women, 42 men, age 40.9±1.3 years, BMI 21.6±0.4 kg m-2), 9% EPS (34 women, 16 men, age 47.1±2.3 years, BMI 24.2±0.7 kg m-2) or 68% overlap group (272 women, 107 men, age 41.4±0.8 years, BMI 22.5±0.3 kg m-2). The majority of patients were female, and the gender distribution was comparable between groups (*P*=0.65). There was a significant difference in age (*P*=0.04) and BMI (*P*<0.003) between the three subgroups, with the EPS group having a higher age and a higher BMI (all *P*<0.05) compared to the PDS and the overlap group. The frequency ratings in the 3 subgroups are shown in Figure 2 and the median frequency scores in Table 1. As expected through the definitions used, the median frequency scores of all dyspeptic symptoms were significantly different in the three subgroups (at least *P*=0.0002). PDS patients had a lower EPS symptom score compared to EPS patients and overlap group patients (all *P*<0.001), while the PDS symptom score was higher in the PDS group and the overlap group compared to the EPS group (all *P*<0.001). EPS patients reported lower bloating and nausea frequency scores than PDS and overlap group patients (both *P*<0.001), with the PDS group reporting less bloating than the overlap group (*P*<0.05). Finally, EPS and PDS patients displayed lower cumulative scores than overlap group patients (*P*<0.001).

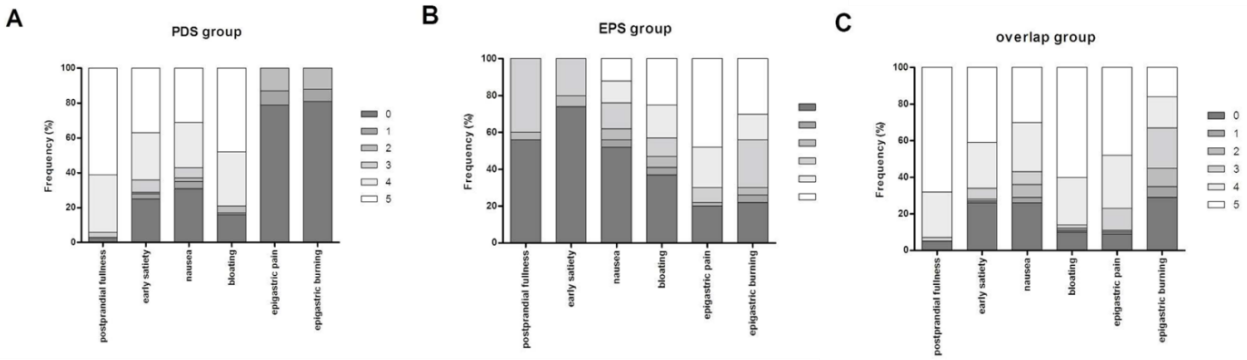

Figure 2. Frequency ratings of dyspeptic symptoms per subgroup. Frequency rating ranges from 0 to 5 (0=absent; 1=once a month or less; 2=two or three times a month; 3=once a week; 4=several times a week; 5=every day). Frequency data are shown as percentages in the (A) PDS group, (B) EPS group and (C) overlap group. EPS=epigastric pain syndrome; PDS=postprandial distress syndrome.

	Symptom	Median frequency (IQR)
PDS (n=131)	PDS score	8.0 (5.0-10.0) *
	EPS score	0.0 (0.0-1.5) *
	Nausea	4.0 (0.0-5.0) *
	Bloating	4.0 (4.0-5.0) *
	Cumulative score	15.0 (12.0-18.0)

EPS (n=50)	PDS score	3.0 (0.0-3.0)
	EPS score	6.0 (5.0-8.3)
	Nausea	0.0 (0.0-3.3)
	Bloating	3.0 (0.0-4.5)
	Cumulative score	13.0 (10.0-15.5)
Overlap (n=379)	PDS score	8.0 (5.0-10.0) †
	EPS score	6.0 (5.0-8.0) ^
	Nausea	4.0 (0.0-5.0) †
	Bloating	5.0 (4.0-5.0) † ^
	Cumulative score	22.0 (18.0-25.0) † ^

Table 1. Median frequency of dyspeptic symptoms in the functional dyspepsia subgroups
Frequency rating ranges from 0 to 5 (0=absent; 1=once a month or less; 2=two or three times a month; 3=once a week; 4=several times a week; 5=every day). Median frequency data are shown as median frequency (IQR). * PDS group significantly different from EPS group, † EPS group significantly different from overlap group, and ^ PDS group significantly different from overlap group after post-hoc test (Dunns). EPS=epigastric pain syndrome; PDS=postprandial distress syndrome.

Gastric sensitivity and gastric accommodation in functional dyspepsia subgroups

Gastric sensitivity and gastric accommodation were studied in a barostat examination in 270 FD patients, including 59 PDS patients, 14 EPS patients and 197 overlap group patients. The proportion of patients who underwent a gastric barostat was significantly lower in the EPS group compared to the PDS and overlap group (*P*=0.004).

Of the total FD study population, 37.4% had a discomfort pressure lower than 6.6 mmHg above the MDP and therefore displayed hypersensitivity to gastric distension. When the prevalence of gastric hypersensitivity to distension was compared between the subgroups, no difference was observed (PDS=30.5%, EPS=21.4% and overlap=40.6%; *P*=0.16; Figure 3A). In addition, there was no statistically significant difference in the median discomfort pressure between PDS patients (8.0 (6.0-12.0) mmHg above MDP), EPS patients (10.0 (7.5-12.0) mm Hg above MDP) and overlap group patients (8.0 (6.0-10.0) mm Hg above MDP) (*P*=0.10; Figure 3B).

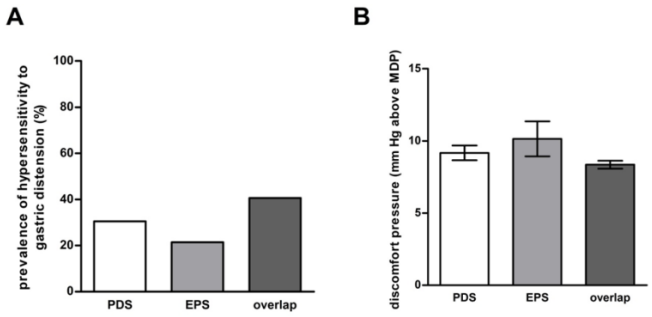

Figure 3. Gastric sensitivity. (A) Prevalence of hypersensitivity to gastric distension in the PDS, EPS and overlap group. (B) Relative discomfort pressure in the subgroups. Data are presented as median (IQR). EPS=epigastric pain syndrome; MDP=minimal distending pressure; PDS=postprandial distress syndrome.

Impaired gastric accommodation (average volume postprandial - preprandial < 64 mL) was present in 36.5% of the FD patients. The prevalence of impaired gastric accommodation in the PDS, EPS and overlap group was 27.6%, 42.9% and 38.7% respectively, with no difference between subgroups (*P*=0.27; Figure 4A). When the postprandial increase in volume was compared between PDS patients (117.6 (55.6-210.4) mL), EPS patients (116.7 (5.8-197.3) mL) and overlap group patients (111.3(10.0-212.6) mL), no statistical significance was observed (*P*=0.23; Figure 4B).

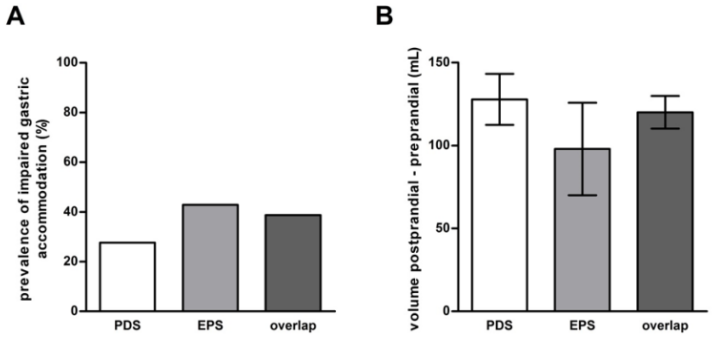

Figure 4. Gastric accommodation. (A) Prevalence of impaired gastric accommodation in the PDS, EPS and overlap group. (B) The difference in mean volume before the meal and mean volume after the meal in the subgroups. Data are presented as median (IQR). EPS=epigastric pain syndrome; PDS=postprandial distress syndrome.

Gastric emptying of solids in functional dyspepsia subgroups

Gastric emptying results were available for 533 FD patients, including 124 PDS patients, 47 EPS patients and 359 overlap patients. The proportion of patients who underwent a gastric emptying test was comparable between subgroups (*P*=0.92). Of the total study population, 22.9% presented with delayed gastric emptying for solids as they had a t1/2>109 min. The prevalence of delayed gastric emptying was comparable between the PDS group (23.2%), the EPS group (14.9%) and the overlap group (23.8%) (*P*=0.39; Figure 5A). There was a significant difference in the t1/2 between PDS patients (82.0 (64.0-107.0) min), EPS patients (72.0 (57.0-94.0) min) and overlap group patients (88.0 (67.5-109.0) min) (*P*=0.02; Figure 5B); with the overlap group having a higher t1/2 (*P*<0.05) compared to the EPS group.

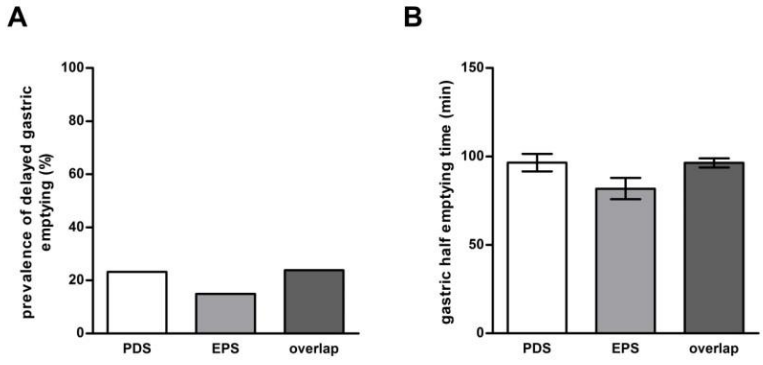

Figure 5. Gastric emptying. (A) Prevalence of delayed gastric emptying in the PDS, EPS and overlap group. (B) The gastric half emptying time in the subgroups. Data are presented as median (IQR). EPS=epigastric pain syndrome; PDS=postprandial distress syndrome.

Association of physiological mechanisms with dyspeptic symptoms

We studied the association between the dyspeptic symptoms and the physiological parameters for gastric sensitivity, gastric accommodation and gastric emptying, using the discomfort pressure threshold, the difference between the postprandial and the preprandial volume, and the t1/2 as independent variables (Table 2). Only relevant associations (n value high enough and acceptable range of symptom scores) were analyzed. After multiple testing correction, the overlap group showed an association between the discomfort pressure and PDS symptoms (*P*=0.03), EPS symptoms (*P*=0.02) and the cumulative symptom score (*P*=0.02); and an association between the t1/2 and nausea (*P*=0.02) and the cumulative symptom score (*P*=0.02). No significant associations were found in the two other subgroups.

Table 2. Correlation of dyspeptic symptoms with physiological parameters in the subgroups n=125 for gastric emptying and n=59 for gastric sensitivity and gastric accommodation in the PDS group, n=47 for gastric emptying and n=14 for gastric sensitivity and gastric accommodation in the EPS group, and n=361 for gastric emptying and n=198 for gastric sensitivity and gastric accommodation in the overlap group. P values < .05 are shown in bold. EPS=epigastric pain syndrome; PDS=postprandial distress syndrome.

	Group Symptom	Discomfort pressure (mmHg above MDP) ρ	OR (95% CI)	P value	Accommodation (mL) ρ	OR (95% CI)	P value	Gastric half emptying time (min) ρ	OR (95% CI)	P value
PDS	PDS score	0.58	(0.20-1.71)	0.63	1.00	(1.00-1.00)	0.88	1.49	(0.69-3.25)	0.63
	EPS score	1.06	(0.31-3.64)	0.95	1.00	(1.00-1.01)	0.65	1.27	(0.48-3.32)	0.90
	Nausea	0.88	(0.33-2.33)	0.95	1.00	(1.00-1.01)	0.57	1.58	(0.74-3.34)	0.60
	Bloating	0.34	(0.11-1.05)	0.22	1.00	(0.99-1.01)	0.36	1.12	(0.51-2.49)	0.95
	Cumulative score	-0.12		0.73	0.05		0.95	0.06		0.82
EPS	PDS score									
	EPS score									
	Nausea							1.20	(0.30-4.87)	0.95
	Bloating							0.48	(0.12-1.91)	0.63
	Cumulative score	-0.30		0.63	-0.08		0.95	1.53	(0.41-5.75)	0.83
								-0.02		0.95
Overlap	PDS score	0.43	(0.23-0.77)	0.03	1.00	(1.00-1.00)	0.83	1.61	(1.04-2.49)	0.17
	EPS score	0.40	(0.22-0.73)	0.02	1.00	(1.00-1.00)	0.22	1.55	(0.97-2.47)	0.22
	Nausea	0.71	(0.41-1.22)	0.60	1.00	(1.00-1.00)	0.95	2.07	(1.31-3.26)	0.02
	Bloating	0.77	(0.42-1.41)	0.71	1.00	(0.99-1.00)	1.00	1.83	(1.10-3.06)	0.12
	Cumulative score	0.24		0.02	-0.0008		0.95	0.17		0.02

2.1.4. Discussion

Because of the heterogeneous nature of FD, it is conceivable that different pathophysiological mechanisms underlie the varied clinical presentations in different subgroups of patients. The aim of this study was to investigate the presence of gastric sensorimotor dysfunction and its possible association with symptom scores in the PDS, EPS and overlap groups as defined by the Rome III criteria. We did not find any differences between the Rome III subgroups in the prevalence of gastric hypersensitivity, impaired gastric accommodation and delayed gastric emptying. The gastric half emptying time, however, was significantly higher in the overlap group compared with the EPS group.

We also observed an association of gastric hypersensitivity with PDS symptoms, EPS symptoms and the cumulative symptom score; and an association of gastric emptying with nausea and the cumulative symptom score in the overlap group. FD is one of the most common gastrointestinal disorders encountered in clinical practice and is defined by the presence of symptoms localized in the epigastric region in the absence of readily identifiable organic abnormalities (41). The available treatment options for this disorder remain unsatisfactory, which is mostly related to the limited knowledge of the pathophysiological mechanisms in FD. To categorize the patient symptom complex more precisely and to simplify the uniformity in defining FD patients for research, the Rome III criteria proposed to subdivide FD into two diagnostic categories: (1) PDS, which is characterized by the presence of early satiety and/or postprandial fullness at least several times per week and (2) EPS, which is characterized by the presence of epigastric pain and/or epigastric burning at least once a week (41). The Rome III document assumed good separation of these entities. Although epidemiological studies demonstrated a good separation between both subgroups (47-51), considerable overlap between PDS and EPS was found in patients seeking medical care, thereby limiting the usefulness of this subdivision in clinical practice (53, 54, 57). The results of the present study confirm the major overlap as 379 of the 560 FD patients reported both PDS and EPS symptoms. An interesting observation was that both PDS and overlap patients reported more bloating and nausea compared to the EPS group. The Rome III document listed upper abdominal bloating and postprandial nausea as symptoms associated to PDS, suggesting that the overlap group clinically more closely resembles the PDS group than the EPS group. The BMI was also higher in the EPS group compared with the PDS and overlap group. This may reflect the presence of early satiety in PDS and overlap patients, which can result in less caloric intake and therefore a lower BMI (22).

It has been argued that FD is a heterogeneous disorder, in which different pathophysiological mechanisms are present in different subgroups of patients and thereby underlie the variations in symptom profiles (62). However, relatively few studies have investigated the differential pathogenesis in the Rome III subgroups. As gastrointestinal sensorimotor dysfunction has been proposed to play a key role in the generation of symptoms in FD patients (170), our goal was to investigate its relationship with the PDS, EPS and overlap group. The prevalence of gastric hypersensitivity, impaired gastric accommodation and delayed gastric emptying in the total FD group of our study was about 37%, 37% and 23% respectively, which is in concordance with previous reports (16). When the prevalence of these pathophysiological factors was compared between the Rome III subgroups, we did not find any difference.

According to the Rome criteria, PDS symptoms are referred to as meal-related, while EPS symptoms are suggested not to be meal-driven (41). In this respect, it seems plausible that PDS might be more associated with impaired gastric accommodation and delayed gastric emptying, and that gastric hypersensitivity would be more prevalent in EPS. However, we did not find evidence for this assumption as our results suggest that PDS and EPS cannot reliably be distinguished based on the pathogenesis. The results of our study only showed a difference in the gastric half emptying time between the EPS group and the overlap group. While Shindo *et al* previously reported slower gastric emptying in PDS patients compared with EPS patients (177), other studies also did not detect an association of delayed gastric emptying with the subgroups (178-180). Furthermore, in agreement with our results, gastric accommodation was shown to be similar between the PDS and the EPS group in earlier studies (179, 180). Di Stefano *et al* however did find a higher prevalence of hypersensitivity in

PDS patients compared with EPS patients (179). In addition, using confirmatory and structural equation modeling, our group previously showed that different mechanisms may play a role in the Rome III subgroups, as they reported an association of gastric sensitivity with PDS and an association of gastric emptying with EPS (181). We do have to mention the low sample size of pure EPS patients that underwent barostat testing for measuring gastric sensitivity and gastric accommodation, which limits the strength of this observation. Because barostat testing is mostly done in patients reporting postprandial fullness and early satiety – and not in patients reporting epigastric pain and epigastric burning – this is less frequently considered in the mechanistic work-up in EPS patients and therefore explains the lower proportion of patients in the EPS group who underwent a gastric barostat.

We also investigated the association of gastric hypersensitivity, impaired gastric accommodation and delayed gastric emptying with dyspeptic symptoms in the Rome III subgroups. Controversy exists about the relationship of impaired gastric accommodation with symptom pattern. Our group reported that impaired gastric accommodation is associated with early satiation and weight loss (22), while other studies did not find an association (60, 171, 173). In the presented cohort, we did not detect a correlation between this pathophysiological factor and any of the symptoms in the different groups. These studies, however, all used a simplified approach to study the association of gastric accommodation with symptom scores. Ly *et al* showed that the use of advanced statistics to model the time course of the gastric accommodation response is more sensitive to detect associations between relevant factors and alterations in gastric accommodation (182). By modeling the entire time curve, they showed an association between gastric accommodation and PDS, but not EPS, symptom severity (182). We did observe an association between gastric hypersensitivity and PDS symptoms, EPS symptoms and the cumulative symptom score in the overlap group. In an earlier study, gastric hypersensitivity was found to be associated with symptoms of belching, postprandial pain and weight loss (61), while smaller studies failed to find such an association (81, 171, 172). Moreover, our group reported associations of gastric sensitivity with nausea and vomiting (181) and with FD symptoms and weight loss (96). Our results also showed a correlation between the gastric half emptying time and nausea and the cumulative symptom score in the overlap group. Some earlier studies reported a high presence of nausea, vomiting and postprandial fullness in patients with delayed gastric emptying (73, 74, 183, 184), while others found no association with symptom pattern (185). The correlations found in this study indicate a contribution of gastric hypersensitivity and delayed gastric emptying to overall symptom scores, but only in the overlap group. A limitation of this study, partially driven by the lower proportion of pure PDS and pure EPS patients in clinic samples, is the low sample size in some subgroups. The latter may explain why associations of high magnitude were sometimes not significant. On the contrary, significant but weak correlations were found in subgroups with high sample size.

Our results suggest that the subdivision of FD patients into PDS and EPS according to the Rome III consensus does not reliably distinguish subgroups with a different pathophysiology, as we only found a higher t1/2 in the overlap group compared with the EPS group. These findings question the relevance of using this classification in clinical practice. Although therapeutic outcomes were not assessed in the present study, our observations also question the guidance of preferentially using prokinetics in PDS and acid suppressive agents in EPS (96). The major obstacle against clinical usefulness is the dominant PDS/EPS overlap group. Overlap can be diminished when the relationship of symptoms to meal ingestion is more rigorously taken into account as shown by a recent analysis from our group (169). In addition, better symptom description, including the use of pictograms, may also allow a more accurate

recognition of the true symptom pattern by the patient, which may also contribute to subdivision in better delineated subgroups (186). The revision of the FD criteria in the Rome IV consensus is in agreement with these ambitions, as it aimed at decreasing the overlap group through new definitions of PDS and EPS that attach more importance to meal-related symptom occurrence (167). Future studies will be needed to evaluate whether this is associated with a better separation of putative underlying pathophysiological mechanisms.

2.2. Rome III Functional dyspepsia subdivision in PDS and EPS: recognizing postprandial symptoms reduces overlap.

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2.2.1. Introduction

Functional gastrointestinal disorders (FGIDs) are highly prevalent conditions with major health and economic impact (43, 187). Functional dyspepsia (FD) is one of the most prevalent FGID, and is defined by Rome III consensus as the presence of symptoms thought to originate from the gastroduodenal region, in the absence of organic disease that is likely to explain the symptoms (41, 42, 62). It has been argued that FD is in fact a heterogeneous condition, with different underlying pathophysiological mechanisms contributing to the symptom pattern (62, 188). The most relevant candidate pathophysiological mechanisms identified to date include impaired gastric accommodation, hypersensitivity to gastric distension and delayed or rapid gastric emptying (16, 62, 188, 189). This heterogeneity is also likely to affect efficacy of therapeutic interventions which target specific mechanisms.

Taking into account this heterogeneity, the Rome III consensus proposed to subdivide FD into Postprandial Distress Syndrome (PDS), characterized by meal-related symptoms such as early satiety and postprandial fullness, and Epigastric Pain Syndrome (EPS) characterized by epigastric burning and epigastric pain. This subdivision was based mainly on expert opinion, and it was proposed to serve as a guide for the diagnostic and therapeutic approach to FD patients (41, 42, 46). In support of the EPS-PDS subdivision, population-based studies found a good separation between PDS and EPS (47-51). In contrast, in clinic samples, overlap of PDS and EPS was found in up to 50% of the patients, and it is evident that this significantly impacts on the usefulness of the subdivision (53-58). In addition, the Rome III subdivision separated belching and nausea from FD symptoms into separate categories of belching and nausea/vomiting disorders (41). Here again, major overlap with EPS and PDS could be found (50, 57, 78, 190). Conceptually, the Rome III subdivision aimed at distinguishing meal-related FD symptoms (PDS) from meal-unrelated FD symptoms (EPS). Through their wording, the PDS symptoms of early satiation during meal intake, and postprandial fullness are inherently linked to meal ingestion. In contrast, the concept of the EPS symptoms of epigastric pain and epigastric burning not being related to meals is not explicitly used in the Rome III definition and questionnaire. However, clinical observations and preliminary questionnaire studies showed that non-PDS symptoms, including epigastric pain and nausea, also occur mainly postprandial in a large subgroup of FD patients (78, 190-192). In studies which quantified symptom occurrence after a standardized meal, postprandial occurring nausea and pain were found in patients with meal-related FD symptoms (190, 191). Hence, taking into account 1) these observations of occurrence of postprandial pain and nausea in presumed PDS patients, and 2) in line with the concept of PDS as “meal-related FD symptoms”, we evaluated whether considering relationship of these symptoms to meal ingestion in FD allows a subdivision with less overlap compared to the current Rome III subdivision.

2.2.2. Materials and Methods

Patient selection

Consecutive ambulatory tertiary-care patients between the ages of 18 and 70 years and presenting with dyspeptic symptoms to the general gastroenterology outpatient clinic or the Neurogastroenterology and motility clinic of the Leuven University Hospital (Belgium) were eligible for the trial. They filled out Rome III gastro-duodenal questionnaires with supplementary questions. These supplementary questions aimed at elucidating the relationship to meal ingestion of non-PDS symptoms such as nausea and epigastric pain, and were developed as part of a previously published study of focus groups in PDS patients, and their content validity was confirmed in cognitive interviews with PDS patients (19). Patients were excluded if the failed to fill out the questionnaire adequately, if they had abnormal findings on upper GI endoscopy, and if they had a history of former upper digestive surgery, diabetes, irritable bowel syndrome, coeliac disease, inflammatory bowel disease or any other symptom of disordered upper GI motility such as dysphagia or globus. In addition, all patients completed a previously validated gastro-esophageal reflux disease (GERD) questionnaire (193, 194). Patients were excluded if they reported frequent and bothersome co-existent GERD symptoms, or if they had a history of reflux esophagitis. FD and the PDS and EPS subgroups were determined by the Rome III criteria.

Rome III based diagnostic categories

In agreement with the Rome III criteria, FD patients were then classified into “pure” PDS if they reported bothersome postprandial fullness and/or early satiation occurring after normal-sized meals at least several times per week during the last 6 months in the absence EPS symptoms. The “pure” EPS subgroup included those patients reporting epigastric pain at least once per week during the last 6 months in the absence of PDS symptoms. A third group was classified as the overlapping EPS-PDS group which comprised patients with both PDS and EPS according to the Rome III criteria.

In a second phase, reclassification of the EPS-PDS overlapping subgroup was done by taking into account non-PDS meal-related symptoms such as postprandial epigastric pain and postprandial nausea. This reclassification was done as a two-step process. First, patients in the EPS-PDS overlapping group reporting postprandial epigastric pain were selected. They were included in the “new PDS” classification if they reported post-prandial epigastric pain at least once a week, and interprandial pain less than once a week. In a second step, the remaining patients in the EPS-PDS overlapping subgroup who reported postprandial nausea several times a week and interprandial nausea less than once a week were also included in the “new PDS” group if they too reported interprandial epigastric pain less than once a week.

Statistics

All FD patients were classified as “pure” PDS, “pure” EPS and overlapping PDS-EPS following the Rome III diagnostic criteria. The occurring frequency of all symptoms was counted and compare between the groups by means of the Fisher’s exact test.

2.2.3. Results

Patient characteristics

Out of a total of 1029 screened patients, 503 presented with organic disease (70% reflux esophagitis, 8% Barrett's esophagus, 2% malignancy, 5% peptic ulcer and 15% other disorders). Out of the 526 patients without underlying organic disorder, only 199 (73% females, 45.5±1.0 years, BMI: 23.9±0.3) fulfilled the ROME III criteria for FD, with symptom onset for more than 6 months ago. The others were eliminated because of a shorter time frame (n=303) or because of predominant reflux symptoms (n=24). Those fulfilling Rome III criteria were subdivided according to the Rome III consensus into "pure" PDS (33%, 70% females, 48.3±1.9 years old, BMI: 24.2±1.1, 14% smoker, 9% daily alcohol intake), "pure" EPS (16%, 66% females, 48.1±2.8 years old, BMI: 23.4±1.0, 9% smoker, 9% daily alcohol intake) and overlapping EPS-PDS (51%, 71% females, 43.7±1.7 years old, BMI: 26.3±0.5, 21% smoker, 11% daily alcohol intake) subgroups (Figure 1).

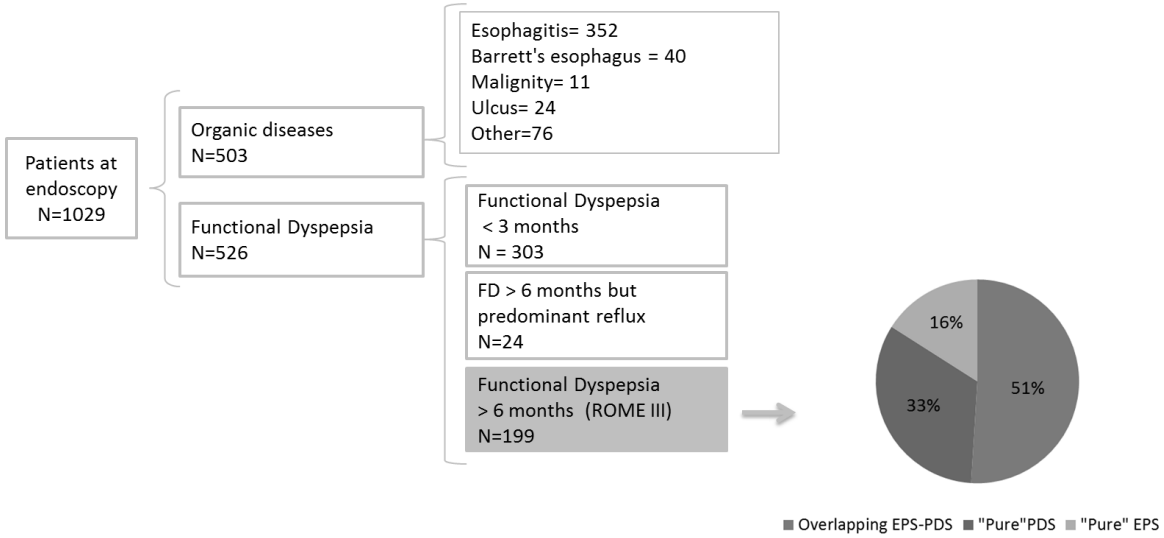
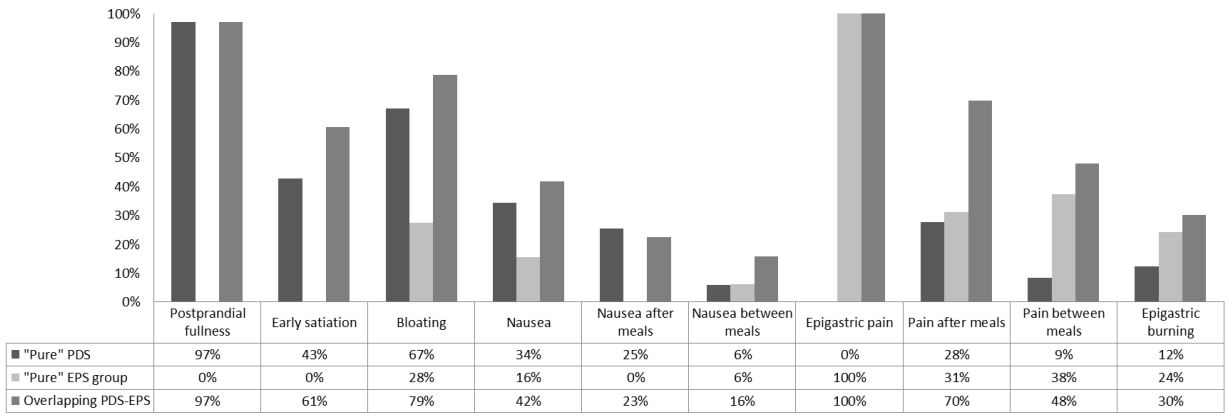


Figure 1. FD patient selection according to ROME III.

Symptom burden in PDS, EPS and the overlapping group according to Rome III

Based on the Rome III questionnaire, the frequency of the symptoms was analyzed for each FD subgroup. In addition, the postprandial nature of a number of non-PDS symptoms was also assessed. This analysis also revealed some discrepancies in patients' symptom ratings: although all pure PDS patients reported epigastric pain less than once a week, 28% of them confirmed experiencing postprandial epigastric pain more than once per week (Figure 2). The symptom occurrence ratings were the highest in the overlapping PDS-EPS patients. These included a high proportion of meal-related symptoms such as postprandial fullness (97%) and early satiation (61%). Moreover, the overlap group reported a high prevalence of postprandial epigastric pain (70%) and postprandial nausea (23%), are reminiscent of the findings in the pure PDS group (Figure 2). Compared to pure EPS patients, the overlapping EPS-PDS patients were characterized by a higher occurrence of postprandial epigastric pain (70% vs. 31%, $p<0.0001$), while the occurrence of epigastric pain between meals was only borderline significantly different (48% vs. 38%, $p=0.05$). In addition, the overlapping PDS-EPS patients reported a higher prevalence of postprandial and interprandial nausea (23% vs. 0% and 16% vs.6%, $p<0.001$). Moreover, the prevalence of upper abdominal bloating was higher in the overlapping EPS-PDS group compared to the EPS group (79% vs. 28%, $p<0.0001$).



	Postprandial fullness	Early satiation	Bloating	Nausea	Nausea after meals	Nausea between meals	Epigastric pain	Pain after meals	Pain between meals	Epigastric burning
"Pure" PDS	97%	43%	67%	34%	25%	6%	0%	28%	9%	12%
"Pure" EPS group	0%	0%	28%	16%	0%	6%	100%	31%	38%	24%
Overlapping PDS-EPS	97%	61%	79%	42%	23%	16%	100%	70%	48%	30%

Figure 2. Symptom characteristics in PDS, EPS and the overlapping group according to ROME III.

Adapted subdivision taking into account postprandial occurrence of symptoms

Taking into account the symptom prevalence findings above, patients in the EPS-PDS overlapping group with exclusively postprandial occurring non-PDS symptoms such as predominant postprandial pain occurring at least once per week and postprandial nausea occurring at least several times a week were reclassified in the PDS group. In this "adapted" subdivision 48% "new" PDS, 16% "new" EPS and 36% "new" overlapping PDS-EPS patients were identified. The symptom profiles in the newly defined groups are shown in Figure 3.The "new" overlapping EPS-PDS group is now characterized by equal occurrences of postprandial and interprandial pain (respectively 57% and 68%) and of postprandial and interprandial nausea (respectively 23 and 20%).

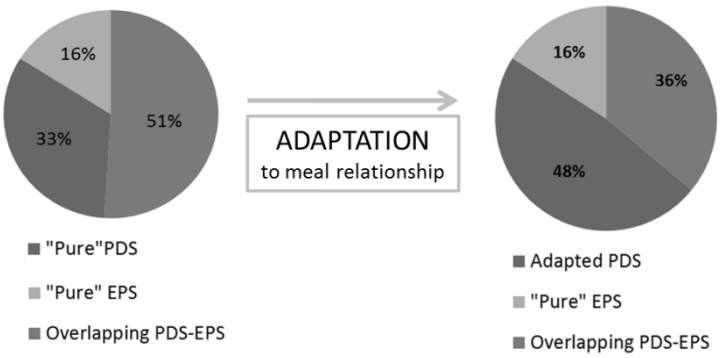


Figure 3. Adapted FD patient distribution

2.2.4. Discussion

FD, defined by the Rome III criteria, is one of the most common gastrointestinal disorders in clinical practice, with a pronounced socioeconomic impact (41, 42). In line with the presumed heterogeneity of FD, the ROME III consensus proposed to subdivide patients into two categories: meal-related PDS symptoms including postprandial fullness and early satiation; and meal-unrelated EPS symptoms characterized by epigastric pain and epigastric burning (41, 42). Although population-based studies found a good separation between PDS and EPS, patient samples showed up to 50% overlap between both, thereby limiting the usefulness of this subdivision in clinical practice (47-51, 53-55).

In the present study, we confirmed the major overlap between EPS and PDS in a group of 199 consecutive newly diagnosed FD patients. We evaluated in detail the relationship of symptoms to meal

ingestion, and this revealed a high proportion of postprandial occurring non-PDS symptoms (epigastric pain and nausea) in the overlapping EPS-PDS group, compared to the pure EPS group.

The postprandial occurrence of FD symptoms has been documented in several studies. Castillo et al. showed that the intensity of symptoms increased after a provocative drinking test in community dyspeptic patients (99). In an epidemiological study from Belgium, dyspeptic symptoms were reported by 20% of the general population and in 37% of the subjects, these symptoms were reported to occur after meals (37%) or after intake of a specific food or beverages (42.6%).

In addition, earlier studies have shown the occurrence of postprandial epigastric pain and postprandial nausea in FD patients. Bisschops et al. studied the occurrence of symptoms after a standard meal in FD patients and healthy controls (78). This study showed that symptoms were induced or aggravated by meal ingestion by the vast majority of FD patients, and this was the case for all symptoms, although epigastric pain, epigastric burning and nausea reached their maximum intensity after the meal compared to fullness or bloating (78).

Vanheel et al. showed the variability of the FD postprandial symptoms intensity over time (191). It was observed that the intensity of fullness, bloating, belching and nausea decreased with the food moving from the stomach into the intestine, suggesting that these symptoms might originate in the stomach (191). The intensity of postprandial epigastric pain and epigastric burning persist with the progression of food into the intestine, suggesting that the stomach as the intestine might play an important role in the origination of these symptoms (191).

Piessevaux et al. linked symptom pattern in FD patients to their gastric distribution of the meal by means of scintigraphy. The results of this study showed that proximal retention and early distal redistribution of a meal could be related to different pathophysiological mechanisms (195).

These results are evidence of the importance of meal-related symptoms in the pathophysiology of FD.

Based on these considerations, we explored an adapted subdivision of FD patients into PDS and EPS subgroups, taking into account the postprandial occurrence of symptoms. Patients in whom epigastric pain and nausea were almost exclusively occurring postprandial were included into the “new” PDS group, which significantly decreased the overlapping group by around one third. While decreasing the overlapping group by itself may be attractive, future studies will need to address whether the pathophysiology and the response to treatment, for instance prokinetic treatment, is similar in the “new” PDS group thus defined.

In conclusion, we confirmed that EPS and PDS symptoms frequently coexist in FD patients, with postprandial occurring symptoms substantially contributing to the overlap. A more rigorous identification of postprandial occurring symptoms, such as postprandial epigastric pain and postprandial nausea, and grouping of these patients in a “new” PDS group, improves the separation between PDS and EPS.

2.3. Analysis of postprandial symptom patterns allows better separation of subgroups of functional dyspepsia patients

2.3.1. Introduction

Functional gastrointestinal disorders are highly prevalent conditions with major health and economic impact (41, 43, 196). Functional dyspepsia (FD) is one of the most frequent functional disorders, and is defined by Rome III consensus as the presence of symptoms thought to originate from the gastroduodenal region, in the absence of organic disease that is likely to explain the symptoms (41, 42, 197). It has been argued that FD is in fact a heterogeneous condition, with different underlying pathophysiological mechanisms contributing to the symptom pattern (16). This heterogeneity is also likely to affect efficacy of therapeutic interventions aimed at a single mechanism.

Taking into account this heterogeneity and based mainly on expert opinion, the Rome III consensus proposed to subdivide FD into Postprandial Distress Syndrome (PDS) and Epigastric Pain Syndrome (EPS) to guide the diagnostic and therapeutic approach of FD patients (42, 46, 47, 53). Conceptually, the Rome III subdivision aimed at distinguishing meal-related FD symptoms (PDS) from meal-unrelated FD symptoms (EPS). PDS symptoms of early satiation during meal intake, and postprandial fullness are inherently linked to meal ingestion. EPS symptoms of epigastric pain or burning are considered meal-unrelated symptoms. However, they can also occur after meals, but their relationship to meals was not explicitly used in the EPS criteria (3). Notwithstanding the good separation between in EPS and PDS in the general population, clinic samples of FD patients display a large overlap between PDS and EPS, which hampers the usefulness of the subdivision (50, 51, 53-55). Clinical observations and preliminary questionnaire studies indicated that an important subgroup of FD patients reports postprandial occurring symptoms of epigastric pain or nausea (58, 78, 190, 191). Previously, we proposed an adaptation on the FD subgroups definition by considering postprandial non-PDS symptoms such as epigastric pain and postprandial nausea part of the “adapted” PDS group, as this generated a better separation of PDS and EPS (169). However, elaborating on this potentially improved subdivision requires more detailed studies of the relationship between symptoms and meal ingestion in the respective groups. Therefore, the aim of this study is to evaluate in detail the relationship of dyspepsia symptoms to meal ingestion in FD patients subdivided according to the Rome III subdivision.

2.3.2. Materials and Methods

Patient selection and general study design

Consecutive ambulatory tertiary-care patients between the ages of 18 and 70 years presenting with dyspeptic symptoms and selected to undergo a gastric emptying breath test were eligible for the trial. Breath tests were done fasting in the morning, without the patient taking drugs that may interfere with gastric emptying rate or epigastric symptom occurrence. Patients were asked to fill out Rome III gastro-duodenal questionnaires with supplementary questions on meal-relationship as previously reported (19), as well as a previously validated gastro-esophageal reflux disease (GERD) questionnaire (193, 194). The supplementary questions consisted of a) a general question about the presence of epigastric pain or discomfort and whether this pain or discomfort is triggered or aggravated by the meal “Do you frequently experience stomach pain or discomfort” (answer: “yes” or “no”), “If yes, does this gastric pain or discomfort is frequently aggravated by the meal” (answer: “yes” or “no”); b) if present, the frequency at which epigastric pain was triggered or aggravated by the meal and “How

often does the gastric pain occurs after a meal?". The answer of this question was related to the frequency of this symptom from 0 "not present", 1 "occasionally", 2 "sometimes", 3 "often", 4 "usually", to 5 "always". The relation of the symptom to the meal was clear when the score was equal or more than 3, c) if present, the frequency at which nausea was triggered or aggravated by the meal "How often does the nausea (urge to vomit) occurs after a meal?". The answer of this question was related to the frequency of this symptom from 0 "not present", 1 "occasionally", 2 "sometimes", 3 "often", 4 "usually", to 5 "always". The relation of the symptom to the meal was clear when the score was equal or more than 3.

The first objective of this study was to explore the validity and accuracy of the supplementary questions to assess and identify non-PDS meal-related symptoms such as postprandial epigastric pain and postprandial nausea. Hypothesis testing in this part of the study evaluated whether the response to these questions allows distinguishing meal-related from meal-unrelated symptoms in a broad population of patients with dyspeptic symptom. The second aim was to explore the meal-relationship of the symptoms and its impact in the different FD subgroups. Here, hypothesis testing focused on the ability of reducing PDS-EPS overlap by taking into account meal-related non-PDS symptoms.

The first analysis was conducted in a larger patient cohort with dyspeptic symptoms, regardless of organic or metabolic co-morbidity. The second analysis was done in the subgroup of FD patients according to the Rome III classification. Patients were classified as FD if they showed normal findings on upper GI endoscopy and did not have a history of former upper digestive surgery, a history of reflux esophagitis, diabetes, predominant irritable bowel syndrome symptoms, coeliac disease, inflammatory bowel disease or any other symptom of disordered upper GI motility such as dysphagia or globus. Patients were also excluded if they failed to fill out the questionnaire adequately.

Prior to filling out the questionnaire, the patients were properly informed about the study and provided witnessed written informed consent. The study was approved by the ethical committee of UZ Leuven in Belgium and was performed in accordance with the Declaration of Helsinki, International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines.

Rome III subgroup classification

All patients were classified subgroups based on the characteristics of their dyspeptic symptoms, using the Rome III criteria. Classification of non-FD patients is not the goal of the Rome classification, but this exploratory step was used to evaluate our first aim. Patients were classified as having "pure" PDS symptoms if they reported bothersome postprandial fullness and/or early satiation occurring after normal-sized meals at least several times per week in the absence of EPS symptoms. "Pure" EPS patients included those patients reporting epigastric pain at least once per week in the absence of PDS symptoms. Finally, the overlapping EPS-PDS subgroup comprised patients with both PDS and EPS symptoms according to the Rome III criteria.

In a second step, the same analysis was performed in patients fulfilling Rome III FD criteria for EPS, PDS or overlapping EPS-PDS. Finally, to evaluate our second aim, patients in the EPS-PDS overlapping subgroup reporting non-PDS meal related symptoms such as postprandial epigastric pain and postprandial nausea occurring often (score equal or more than 3) were reclassified into the "adapted" PDS group as previously reported (169).

Gastric emptying breath test

The gastric emptying breath test is a standard diagnostic tool to measure gastric emptying rate in patients with dyspeptic symptoms (198). After an overnight fast, patients ingested a standardized solid meal that consisted of 60 g of white bread, an egg, the yolk of which was doped with 74 kBq of 14C octanoid acid sodium salt (DuPont, NEN Research, Boston, MA, USA) and 300 ml of water in which 100 mg 13C glycin (99% enrichment; Isotec, Miamisburg, OH, USA) was dissolved. The meal was consumed within a five minute period. The total caloric value of the test meal was 250 kcal. After eating, patients gave a breath sample and scored the severity (0: absent - 4: very severe) of 6 epigastric symptoms (fullness, bloating, nausea, epigastric pain, burning, and belching) every 15 minutes until 4 hours postprandial. The breath samples were collected in sample tubes and GE rate was analyzed by determining the radiation by liquid scintillation counting (Packard Tri-Carb Liquid Scintillation Spectrometer, model 3375, Packard Instrument Company, Downers Grove, IL, USA). Delayed gastric emptying is defined as a half emptying rate (T ½) of more than 109 minutes and accelerated gastric emptying is defined as a half emptying rate (T ½) of 30 minutes or less.

Data analysis

Patients were subdivided into EPS and PDS subgroups according to the Rome III classification, or an adapted classification based on meal-relationship of non-PDS symptoms, as previously reported (18). The severity of meal-related dyspeptic symptoms was defined as the sum of the severity scores recorded during the breath test after the ingestion of a standardize meal for fullness, epigastric pain, epigastric burning, nausea and bloating.

The cumulative meal-related dyspepsia symptom severity was compared between groups using Mann–Whitney test for non-parametric analysis of unpaired non-normally distributed data. Non-parametric Spearman correlation was used to study the relationship between the reported frequency of a symptom and the severity of that symptom after the breath test. The time course of the severity of the symptoms was studied and correlated to the meal-relationship characteristics determined by the supplementary meal-related questions. Mean severity scores over time of the symptoms were compared by the student's t-test. In addition, the relation between the severity of dyspepsia symptoms and the gastric emptying rate was also evaluated.

2.3.3. Results

Patient population with dyspepsia symptoms

A total of 169 patients (67% females, 44.9±1.2 years old and a BMI of 27.7±2.2 $Kg.m^2$) presenting with dyspeptic symptoms who were referred for a gastric emptying breath test participated in the study. In this population, 22% were smokers, 7% consumed alcohol at daily bases and 6% took NSAIDs. Based on endoscopy findings, patient history and additional testing, 64% of the patients were identified as FD patients. Excluded from the FD cohort were patients with reflux esophagitis or Barret's esophagus at endoscopy, a history of prior digestive surgery, diabetes, predominant IBS or an esophageal motor disorder, etc. (Table 1).

Table 1. Overview of the different diagnoses on the patient population referred for a gastric emptying breath test.

Diagnosis	Number	%
Functional dyspepsia	110	64%
Reflux – Esophagitis	14	8%
Digestive surgery	12	7%
Esophageal motility disorder	10	6%
Predominant IBS	7	4%
Diabetes	7	4%
Barrett's esophagus	2	1%
Other diseases	11	7%
Total number of patients	**169**	

When subdividing the total population of patients with dyspeptic symptoms, applying the Rome III subgroup criteria for FD, 18% were characterized by pure PDS symptoms, 7% by pure EPS symptoms and 64% showed overlapping PDS and EPS. A number of patients (11%) could not be subdivided according to the Rome III criteria based on the frequency or characteristics of their epigastric symptoms. The solid gastric emptying test showed a mean half gastric emptying rate of 89.9±4.5 min, with maximal range of 300 min and a minimum of 24 minutes. Gastric emptying rate was delayed in 23% of the patients and accelerated in 3% (Table 2).

Table 2: Overview of symptom pattern in patients with dyspeptic symptoms that participated in the study

Dyspeptic symptom	**Dyspepsia patients**
Postprandial fullness	80%
Early satiation	56%
Upper abdominal bloating	73%
Nausea	47%
Postprandial nausea	42%
Epigastric pain	66%
Postprandial epigastric pain	47%
Epigastric burning	33%
Excessive belching	60%
Heartburn	49%
Delayed gastric emptying rate ($T_{1/2}$<109 min)	23%

Meal-related symptoms association

Eighty-nine percent of the patients reported suffering from epigastric pain or discomfort, and 66% of the patients reported these symptoms to be aggravated or originated by the meal. Patients reporting that symptoms are aggravated by the meal ("yes" to the first supplementary question) tended to have a higher meal-related cumulative dyspepsia severity score after a standardize meal (57.1±6.1 vs. 76.3±5.6; non-parametric Mann-Whitney test: p=0.06).

The frequency of the symptoms recorded by the Rome III questionnaire correlated better with the severity of the symptoms recorded after the standardized meal during the breath test when patients reported the symptoms to be aggravated by the meal ("yes" to the first supplementary question) (Table 3).

The aggravation of nausea or epigastric pain after the meal ("yes" to the second and third supplementary questions) also showed higher meal-related cumulative nausea and epigastric pain recorded during the breath test (non-parametric Mann-Whitney test for postprandial nausea: 23.6±2.2 vs. 6.8±1.3, p<0.0001 ; for postprandial pain: 24.1±2 vs. 11±1.5, p<0.0001).

Finally, the half emptying time showed a modest correlation with the total dyspepsia symptom score reported during the gastric emptying test (r=0.23, p=0.003). A moderate correlation with gastric empting rate was found only for the individual symptom severities of postprandial fullness (r=0.23, p=0.003) and upper abdominal bloating (r=0.22, p=0.004).

Table 3: Non-parametric Spearman correlation between frequencies of symptoms scored on the Rome III questionnaire and the severity of symptoms score during the gastric emptying test in the entire patient population. The patient cohort was subdivided into depending their answers to the first supplementary question "does this gastric pain or discomfort is frequently aggravated by the meal?" *NS: no significant (p>0.05)*

Answer first supplementary question	**"Yes"**		**"No"**	
Severity vs. Frequency	**R**	**p-value**	**R**	**p-value**
Fullness	0.52	<0.0001	0.54	<0.0001
Bloating	0.46	<0.0001	0.57	<0.0001
Nausea	0.63	<0.0001	0.32	0.02
Epigastric pain	0.57	<0.0001	0.31	0.02
Epigastric burning	0.45	<0.0001	0.41	0.002
Belching	0.61	<0.0001	0.38	0.003

FD patients

Demographics

A total of 110 patients were diagnosed with functional dyspepsia. In this population 21% were smokers, 7% consumed alcohol at daily bases and 5% took NSAIDs. A number of patients (n=14; 13%) could not be subdivided as FD as per Rome criteria due to lack of occurrence of the epigastric symptoms as defined by the Rome criteria. When subdividing the FD population as per Rome III subgroup criteria (n=96), 9% of the patients were classified as EPS alone, 30% as PDS alone and 61% as overlapping EPS and PDS. The characteristics of these groups are summarized in Table 4.

The results of the solid gastric emptying test on FD patients showed a mean half gastric emptying rate of 90.4±4.9min. Gastric half emptying was delayed in 18% of the patients and accelerated in 3%. For PDS patients, mean half gastric empting time was 85.2±5.1 minutes (14% delayed), 75.4±7.0 minutes (0% delayed) for the EPS subgroup and 90.4±5.9 min (23% delayed) for the overlap patients. One Way Anova - Kruskal Wallis test did not show significant differences between the half gastric emptying rate of these subgroups (p=0.25)

Table 4: Symptom characteristics of FD patients as a group, and in the different dyspepsia subgroups.

Dyspeptic symptom	FD (n=110)	PDS (n=29)	EPS (n=9)	Overlap (n=58)	Other FD (n=14)
Age (years old)	44.4±1.6	40±2.9	46.2±5.9	45.4±2.2	48.3±4.4
Gender (% females)	72%	69%	89%	61%	89%
BMI ($Kg.m^2$)	27.3±2.5	24.5±1.1	25.5±1.6	29.2±4.5	26±1.1
Postprandial fullness	79%	100%	0%	100%	0%
Early satiation	55%	62%	0%	72%	0%
Upper abdominal bloating	72%	93%	0%	86%	14%
Nausea	53%	55%	44%	62%	14%
Postprandial nausea	27%	31%	0%	36%	0%
Epigastric pain	62%	0%	100%	100%	0%
Postprandial epigastric pain	45%	0%	0%	71%	0%
Epigastric burning	31%	24%	56%	38%	0%
Excessive belching	56%	54%	44%	62%	36%
Heartburn	45%	38%	78%	59%	29%
Delayed gastric emptying rate ($T_{1/2}$<109 min)	18%	14%	0%	23%	33%

Meal-related symptom association in FD

Sixty-five percent of the FD patients reported the symptoms to be aggravated or triggered by the meal ("yes" to the first supplementary question). The meal-related symptom score was significantly higher in this group (75.9±9.1 vs. 107.3±8.3; p=0.02).

Following the reported aggravation of symptoms by the meal, the frequency scores of the symptoms reported on the Rome III questionnaire correlated better with the symptom severity score recorded after the standardized meal during the breath test in the FD population. In the subgroup of patients that reported no symptom worsening after meals, nausea, epigastric pain and belching showed no correlation to the severity scores after the meal (Table 5).

The additional second and third supplementary questions on meal-related nausea and epigastric pain showed increased symptoms severity scores of nausea and pain recorded after the standardized meal during the breath test (non-parametric Mann-Whitney test for postprandial nausea: 24.7±2.8 vs. 6.8±1.9, p<0.0001 ; for postprandial pain: 25.4±2.8 vs. 10±2.1, p<0.0001). The half emptying time rate was not correlated with the total dyspepsia symptom score reported by the FD patients during the gastric emptying test (r=0.15, p=0.14).

Table 5: Association between frequency score on the Rome III questionnaire and the severity score during the gastric emptying test in the FD population. FD patients were subdivided into depending their answers to the first supplementary question "does this gastric pain or discomfort is frequently aggravated by the meal?" *NS: no significant (p>0.05)*

Answer first supplementary question (FD)	**"Yes"**		**"No"**	
Severity vs. Frequency	R	p-value	R	p-value
Fullness	0.43	0.0002	0.51	0.005
Bloating	0.41	0.0005	0.53	0.004
Nausea	0.69	<0.0001	0.34	NS, 0.08
Epigastric pain	0.64	<0.0001	-0.12	NS, 0.56
Epigastric burning	0.68	<0.0001	0.53	0.004
Belching	0.54	<0.0001	0.33	NS, 0.09

During the breath test, the symptom scores in functional dyspepsia (FD) patients were measured every 15 minutes before (time point 0) and for 240 min after ingestion of a standard meal. Immediately after the meal, all symptoms increased compared to baseline (time 0 vs. time 5). Bloating, postprandial fullness and belching increased rapidly after meal ingestion to reach a peak intensity at time point 30 min for bloating (max score=1.36) and for postprandial fullness (max score=1.7) and 45 min for belching (max score= 1.09) followed by a gradual decrease.

The elevated symptom intensity score was maintained until the end of the measurement period for epigastric pain (max score reached at 60-75 min=1.09), nausea (max score reached at 75 min=1.03) and burning (max score reached at 105 min=0.99).

The area under the curve was highest for fullness (343.2±11.6) followed by bloating (320.7±12.8), epigastric pain (317.4±19.1), nausea (306.1±18), burning (300.1±19.6) and belching (300±17.7). Severity scores increase directly after the ingestion of the standardized meal. Bloating, fullness and belching reach maximal score 30 min after the meal, followed by a decrease. Epigastric pain, nausea and epigastric burning reach a maximum after one hour and maintained the score during the test.

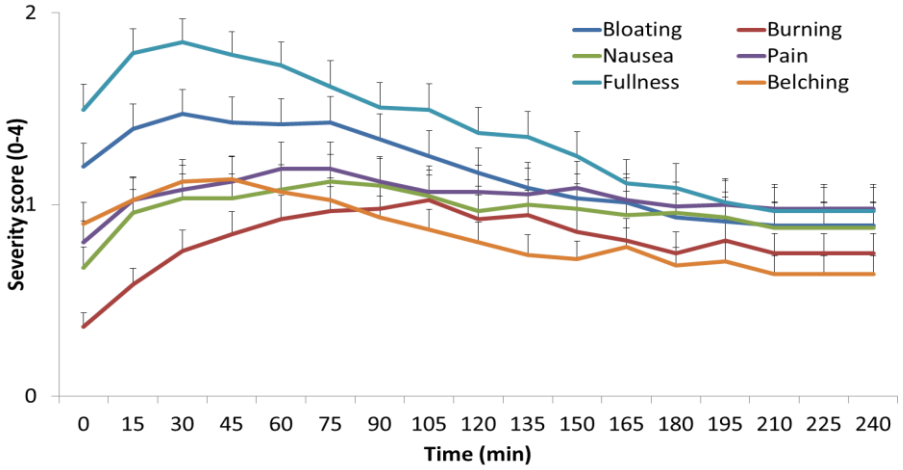


Figure 4.Time Course of FD symptom severity scores during the gastric emptying test in all FD patients.

Meal-related symptom association in PDS, EPS and overlap PDS-EPS subgroup

Most of the patients in "pure" PDS and overlap subgroup reported the symptoms to be aggravated by the meal (Table 6).

Table 6: Answer to the supplementary meal-related questions.

Supplementary questions	1.Aggravate by the meal	2.Postprandial nausea	3.Postprandial epigastric pain
PDS	79%	45%	28%
EPS	44%	33%	0
Overlap PDS-EPS	72%	57%	71%

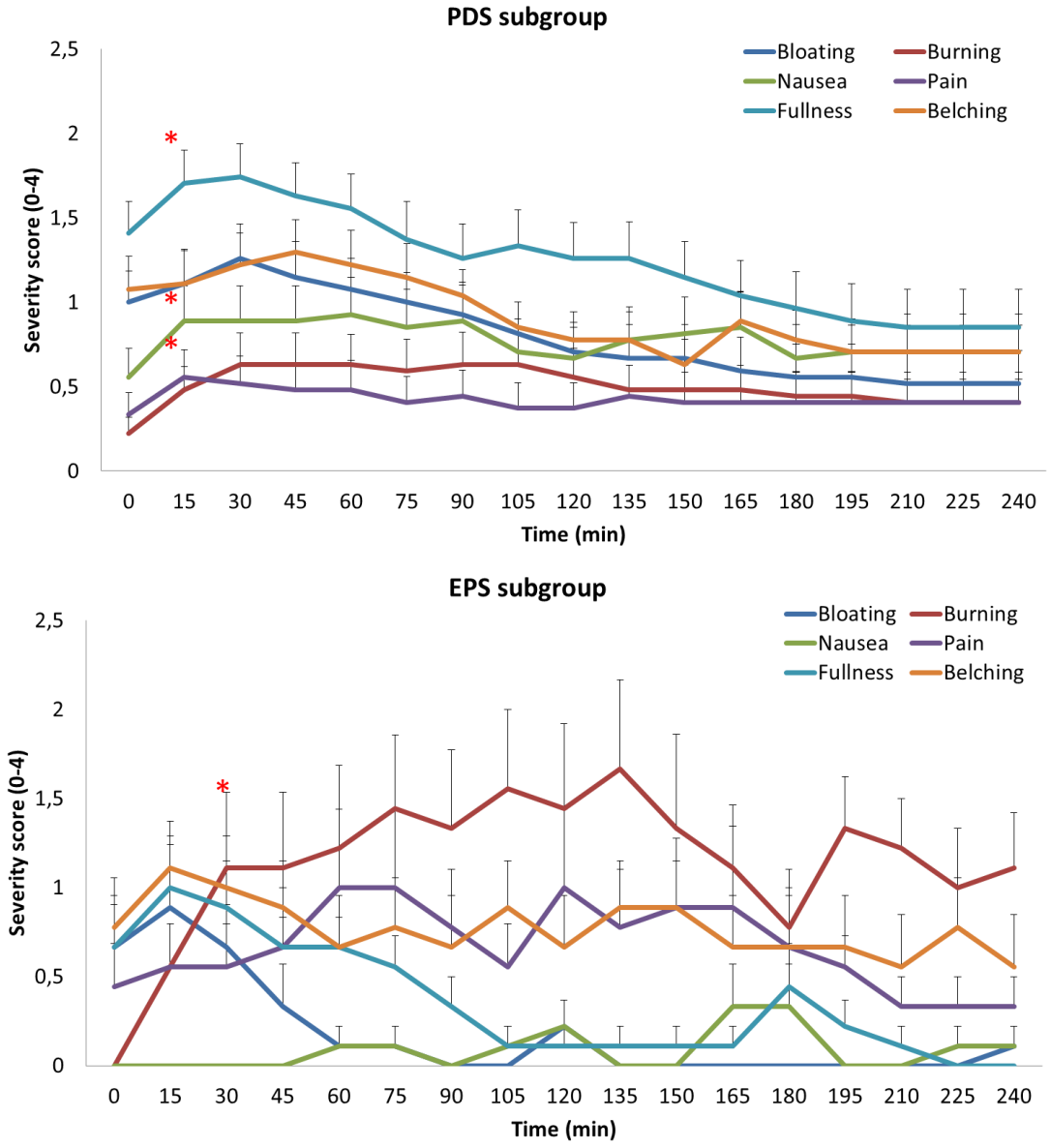
The overlap PDS-EPS subgroup symptom frequency as defined by the Rome III tended to show a good association to all symptom severity assessed after the breath test standardized meal. In the PDS subgroup, the frequency of bloating associated poorly to the severity occurrence of bloating after the meal (r=0.14; p=0.46). In the EPS subgroup, none of the symptoms reported on the Rome questionnaire with additional questions associated well with the severity scores during the breath test (Table 6). The result of the breath test showed a modest correlation between the half emptying time and the cumulative dyspepsia severity score in the overlap subgroup (r=0.36; p=0.006). Nausea is generally associated to delay gastric emptying in idiopathic gastroparesis (199). In this study, the severity of nausea during the breath test also showed a modest correlation (r=0.31, p=0.02) to the breath test results. For PDS, however, no significant association was observed for the half gastric emptying time and the cumulative dyspepsia symptoms (r=-0.29; p=0.14) or nausea (r=-0.14; p=0.49). No correlation was found for the cumulative symptoms and breath test results (r=0.16; p=0.68) in the EPS subgroup.

The time course of the severity of the symptoms during the breath test was compared between the different subgroups. The EPS subgroup showed the highest scores for epigastric burning, starting 30 min after the meal to a maximal severity score of 1.6±0.5 at 135 min. Epigastric pain (mean severity score 0.6±0.06; max=1 at 60 min) and belching (mean severity score 0.7±0.04, max=1.1 at 15 min) did not differ significantly from baseline and were similar over the entire measurement time. Bloating and fullness increased slightly to a maximum 15 min after the meal (max=0.9 and 1, respectively), but did not differ significantly from baseline, and decreased significantly below baseline severity score. Little nausea was reported over time (Figure 5).

The PDS and the overlap group showed similar symptom patterns. In the PDS subgroup, fullness, epigastric pain and epigastric burning increased significantly from baseline after the meal. Bloating increased slightly but not significantly after the meal. The severity of fullness, bloating and belching decreased gradually (Fullness= maximal score 1.7 at 30 min; Bloating= maximal score 1.3 at 30 min; Belching= maximal score 1.3 at 45 min). At the end of study (after 3h30min), only the severity of fullness decreased below baseline (p=0.03). The severity scores of epigastric pain, nausea and burning persisted until the end of the study (Pain= mean severity score 0.5±0.06, max=0.55 at 15 min; Nausea= mean severity score 0.7±0.04, max=0.9 at 60 min; Burning= mean severity score 0.5±0.03, max=0.63 at 30 min) (Figure 5).

Compared to baseline, in the PDS-EPS overlap subgroup, the severity score of the symptoms was significantly increased after the first 15 min for all symptoms, except for belching which never reached

a significant difference from baseline. Moreover, fullness (maximal score 2.1 at 30 min), bloating (maximal score 1.9 at 75 min) and belching (maximal score 1.1 at 45 min) reached their maximum severity scores early on and decreased afterwards. After reaching its maximal severity, the score of epigastric pain, nausea and epigastric burning severity remained high until the end of the study (Pain max severity score=1.6 at 75 min; Nausea max severity score=1.4 at 75 min; Burning max severity score=1.1 at 105 min) (Figure 5).



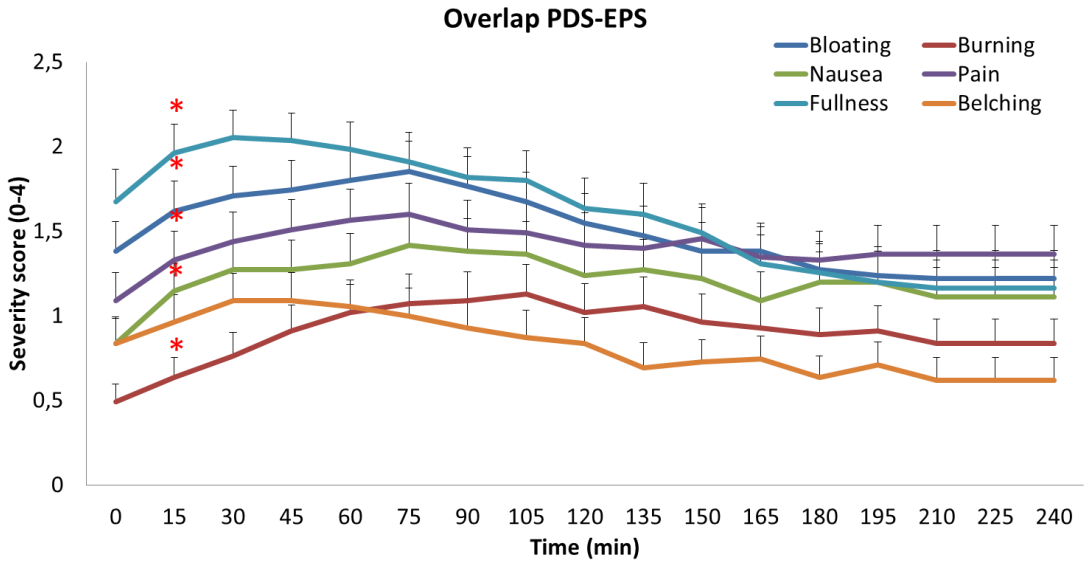


Figure 5. Time course of the severity score of PDS, EPS and overlap PDS-EPS dyspepsia symptoms after a standardized meal (244 Kcal) during the gastric emptying breath test. *p<0.05.

Meal-related symptom association in "adapted" PDS and overlap PDS-EPS subgroup

Based on the frequency of non-PDS symptoms such as postprandial epigastric pain in the overlap PDS-EPS subgroup, patients were redistributed to the PDS population as previously described (169). Following this re-allocation, the "adapted" PDS population included 70 patients (64%; 77% females, 43.1±2.1 years old, BMI 27.4±3.5 Kg.m^2) and the overlap subgroup was reduced to 17 patients (15%; 53 % females, 45.9±3.7 years old, BMI 29.2±6.6 Kg.m^2). The pure EPS subgroup was not altered (8 %)(Figure 6).

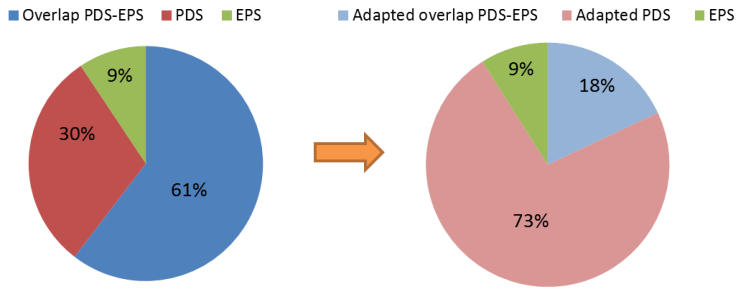


Figure 6. Comparison of FD subdivision before and after the adaptation of the PDS subgroup. Left: FD subgroups as per Rome III criteria. Right: "Adapted" PDS and PDS-EPS overlap subdivision: postprandial fullness and /or early satiation more times and postprandial epigastric pain at least once a week. EPS and overlap PDS-EPS: epigastric pain with no postprandial epigastric pain at least once a week.

The association of symptom frequency with the meal-related symptoms after the standardized meal during the gastric emptying test continued to be present in the "adapted" PDS subgroup. This was now found to be less prominent in the "adapted" overlap subgroup; now showing only good correlations with nausea, epigastric burning, belching and heartburn (Table 7). The results of the half emptying

time compared to the sum of dyspepsia symptoms were not significantly altered for the "adapted" PDS subgroup (r=0.16; p=0.17), or for the "adapted" overlap PDS-EPS subgroup (r=0.27; p= 0.29).

Table 7. FD subgroups: Rome III symptom frequency correlated with the sum of symptom severity after a standardized breath test meal in the different subgroups and in the "adapted" subdivision.

FD subgroups			**Adapted FD subgroups**		
Overlap subgroup (n=58)	**R**	**p-value**	**Adapted overlap Subgroup (n=17)**	**R**	**p-value**
Fullness	0.45	0.0004	**Fullness**	0.45	NS, p=0.07
Bloating	0.47	0.0002	**Bloating**	0.26	NS, p=0.31
Nausea	0.58	<0.0001	**Nausea**	0.48	0.05
Epigastric pain	0.39	0.003	**Epigastric pain**	-0.08	NS, p=0.73
Epigastric burning	0.73	<0.0001	**Epigastric burning**	0.54	0.03
Belching	0.56	<0.0001	**Belching**	0.52	0.03
PDS subgroup (n=29)	**r**	**p-value**	**Adapted PDS subgroup (n=70)**	**R**	**p-value**
Bloating	0.14	NS, p=0.46	**Bloating**	0.37	0.0017
Nausea	0.68	<0.0001	**Nausea**	0.61	<0.0001
Epigastric pain	0.13	NS, p=0.51	**Epigastric pain**	0.59	0.0001
Epigastric burning	0.43	0.02	**Epigastric burning**	0.69	0.0001
Belching	0.38	0.04	**Belching**	0.50	0.0001
EPS subgroup (n= 9)	**r**	**p-value**	**EPS subgroup (n= 9)**	**R**	**p-value**
Bloating	0.57	NS, p=0.12	**Bloating**	0.57	NS, p=0.12
Nausea	0.29	NS, p=0.44	**Nausea**	0.29	NS, p=0.44
Epigastric burning	-0,19	NS, p=0.64	**Epigastric burning**	-0,2	NS, p=0.64
Belching	0.37	NS, p=0.31	**Belching**	0.37	NS, p=0.31

The time course of the severity of the symptoms during the breath test in the "adapted" PDS subgroup continued to show a relationship to the ingestion of the meal. Compared to baseline, the severity score of the symptoms was significantly increased for all symptoms, except for belching. Immediately after the meal fullness, bloating and belching reached maximal severity score and this was followed by a decrease below baseline (Fullness= maximal score 2.1 at 30 min,; Bloating= maximal score 1.6 at 30 min; Belching= maximal score 1.2 at 45 min). Epigastric pain, nausea and epigastric burning reached maximal severity and this remained elevated until the end of the study (Pain max severity score=1.4 at 60 min; Nausea max severity score=1.3 at 75 min; Burning max severity score=1 at 105 min).

In the "adapted" overlap group, the severity of the symptoms was only significantly increased for fullness, epigastric burning and nausea compared to baseline. Fullness, bloating and belching reached maximal severity score and this was followed by a decrease below baseline (Fullness= maximal score 1.4 at 60 min; Bloating= maximal score 1.3 at 75 min; Belching= maximal score 1.1 at 15 min). Epigastric pain increased gradually until its maximal score (1.1 at 240 min), nausea and epigastric burning reached maximal severity and remained quite high until the end of the study (Nausea max severity score=1 at 90 min; Burning max severity score=0.8 at 105 min).

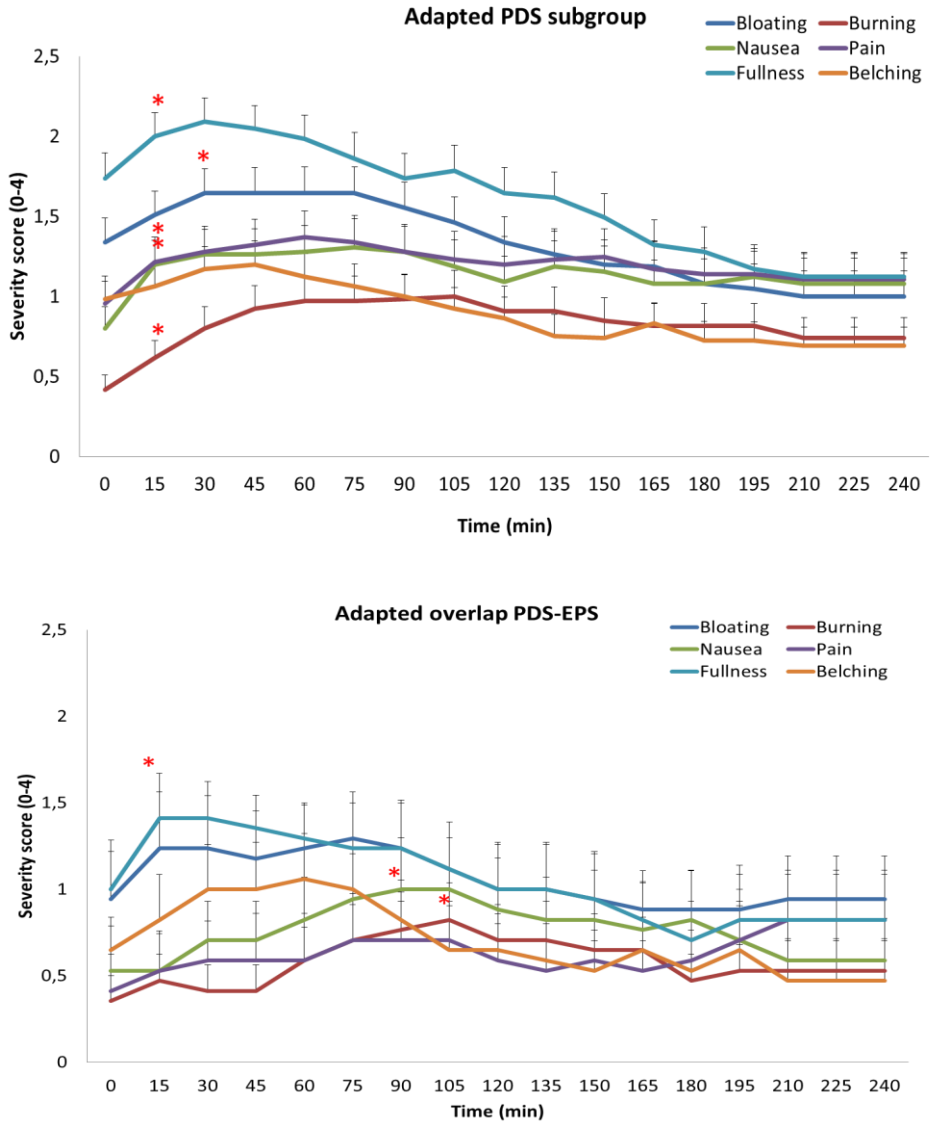


Figure 7.Time course of the "adapted" subdivision of PDS patients and the overlap PDS-EPS subgroup

2.3.4. Discussion

The Rome III subdivision aimed at distinguishing patients with meal-related FD symptoms (PDS) from those with meal-unrelated FD symptoms (EPS). However, in patients coming to medical attention, a large overlap between both groups hampers the usefulness of the subdivision (50, 51, 53-55). Previously, we have reported that an important subgroup of FD patients reports non-PDS symptoms which are mainly occurring postprandial, such as postprandial epigastric pain, and that the recognition

of the meal-related nature of these symptoms may help to classify FD patients outside the overlap subgroup (58, 78, 169, 190, 191).

In the present study we aimed to further evaluate the gain of identifying the relationship of individual dyspepsia symptoms to meal ingestion by assessing its relationship to systematically measured meal-related symptoms. This analysis was first performed in a general, unselected, population of patients undergoing gastric emptying testing for dyspeptic symptoms to establish the validity and accuracy of the questions to detect meal related symptoms, quantified as the severity score of 6 epigastric symptoms reported after the ingestion of a standardized meal during a standard diagnostic gastric emptying breath test. In a second phase, we used the same approach to study the relationship of the meal and dyspepsia symptoms in the originally defined and "adapted" subgroups of FD patients.

A positive response to the question whether symptoms were triggered or aggravated by a meal identified a FD subgroup with higher symptom severity score after a standardized meal, confirming the validity of this question. A similar tendency was also observed in the general population of dyspeptic patients, suggesting that this additional question helps to discriminate FD patients with meal-related symptoms and may improve, therefore, the selection of "adapted" PDS patients. In both populations, the reported frequency of dyspepsia symptoms in the Rome III questionnaire and additional meal-related questions correlated well with the symptoms recorded after the standardized meal during the breath test, suggesting that patients adequately recall the timing of their symptoms and that the questionnaire is able to accurately assess this information.

After subdividing FD patients into the different subgroups, it was observed that the frequency of symptoms in the PDS and the overlap PDS-EPS group correlated well with the meal-related symptom intensities scored after ingestion of the standardized meal. In this group, most of the dyspepsia symptoms peaked immediately after the meal, with the exception of bloating in the PDS subgroup. Bloating is frequently associated with IBS. The Rome criteria includes bloating as a symptom that may coexist in FD provided that these symptoms are not being relieved by bowel movement (197). In the present study patients did report bloating symptoms as frequent and bothersome symptom, but patients with predominant IBS symptoms were excluded.

In the EPS subgroup no associations were found between the Rome questionnaire and the additional meal-related questions on the one hand, and the postprandial symptom severity scores on the other hand, confirming the lack of relationship of pure EPS symptoms to the meal. In keeping with this notion, the symptom severity profile showed clear differences from the PDS and overlap subgroups suggesting a different pathophysiological background for symptom generation in these respective subgroups. While PDS is mainly considered a disorder of motor control, EPS has been related to gastric and duodenal (acid, mechanical distention) hypersensitivity (30, 61, 194, 200, 201), *H. pylori* infection (202), low-grade inflammation and altered mucosa permeability (203-206).

We have previously suggested an adaptation of the Rome subgroups definition by considering frequent postprandial epigastric pain as part of the PDS subgroup in order to reduce the overlap (169). Furthermore, the Rome consensus has recently updated the Rome diagnostic criteria (Rome IV), now recognizing the occurrence of postprandial pain and nausea as part of PDS (197). In the present study, 53% of the FD patients had overlapping PDS-EPS symptoms, and by revising the subdivision as previously described the overlap decreased to 15%. In the revised overlap subgroup, the relation of meal-related symptom severity with the symptoms reported in the Rome questionnaire became less

clear, and only the severity of nausea and epigastric pain increased significantly from baseline one hour after the meal.

Taken together, our data support the hypothesis that FD symptoms are generated differently in EPS compared to “adapted” PDS patients. Previously, *Vanheel et al*. demonstrated that the time course of dyspeptic symptom generation is probably related to the location of the meal in the gastrointestinal tract (the gastric and the intestinal phase) (191). In line with this concept, we can propose from our data that symptoms in the “adapted” PDS population are originating from the stomach, while symptoms in the EPS and the “adapted” overlap population may originate from the duodenum.

Finally, in the last years, the relationship between FD and idiopathic delayed gastric emptying has been a topic of intense debate. Both conditions share symptom pattern, pathophysiological alterations and a therapeutic approach with prokinetic drugs (63, 97, 207). In the present study, however, the severity of symptoms was inconsistently and poorly associated to the gastric emptying rate in FD as a group or in the different subgroups, suggesting that delayed gastric emptying is not the primary cause leading to meal-related symptoms.

In conclusion, this study confirms that the meal plays an important role in the triggering or aggravation of symptoms in FD, especially in the PDS subgroup and in a large part of the overlap PDS-EPS subgroup. Additional questions on meal-related symptoms are accurate and help to identify patients in the PDS-EPS group with meal-induced symptoms. Finally, adaptation of the subdivision, taking into account the relationship of the symptoms to the meal, helps reduce the overlap between EPS and PDS, with a proportionate increase of the PDS group. The symptoms of EPS patients and of the remaining patients in the overlap subgroup show a less clear link to the meal suggesting a different pathophysiological mechanism.

Chapter 3

Development and validation of PRO questionnaires

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3.1.1. Introduction

Functional dyspepsia (FD) is defined by the Rome III consensus as the presence of at least one of four cardinal dyspepsia symptoms (postprandial fullness, early satiation, epigastric pain and epigastric burning,) in the absence of organic or metabolic disturbances likely to explain those symptoms (41). FD is the main cause of upper gastrointestinal symptoms in the general population, affecting 5-15% of adults, with considerable quality of life and health-economic impact (42, 43).

In order to optimize the diagnostic and therapeutic approach of this disorder, the Rome III consensus subdivided FD patients into two subgroups: epigastric pain syndrome (EPS) and postprandial distress syndrome (PDS), based on the assumption that these have different underlying pathophysiology and may require different initial treatment strategies (41, 42, 46, 96). However, the heterogeneity of the disorder and the great overlap between FD subgroups poses considerable challenges to managing symptoms in FD patients (42, 96, 169).

In most FD questionnaires, including the Rome III diagnostic questionnaire, FD diagnostic criteria are mainly driven by the frequency of symptoms, both for the PDS subgroup (postprandial fullness or early satiation at least several times per week), and for the EPS subgroup (epigastric pain or epigastric burning at least once per week) (42). Severity is not addressed, which seems to suggest it is a less important parameter. However, it is unclear to which extent frequency and severity of symptoms in FD are closely correlated or whether they are unrelated.

The aim of this study was to explore the frequency and severity of dyspepsia symptoms, and the relationship between both, in FD patients, taking into account the Rome III subdivision into EPS and PDS subgroups.

3.1.2. Materials and methods

Patient selection

Consecutive ambulatory FD patients presenting to the gastroenterology outpatient clinic at the University Hospitals Leuven (Belgium) and fulfilling the ROME III diagnostic criteria were recruited for this study. Patients were excluded if they had abnormal findings on upper GI endoscopy, if they failed to adequately fill out the questionnaire, if they had a history of former upper digestive surgery, diabetes, irritable bowel syndrome, coeliac disease, inflammatory bowel disease or if they presented frequent and bothersome typical GERD symptoms such as heartburn or regurgitation.

Subdivision of FD subgroups

The patients completed a specific questionnaire that evaluated both the frequency and severity of eight FD symptoms: early satiation, postprandial fullness, epigastric pain, epigastric burning, upper abdominal bloating, nausea, vomiting and belching. The severity of the symptoms was graded 0-3 according to symptom impact on patients' daily activities; 0: absent, 1: mild, 2: moderate (not interfering with daily activities), 3: severe (interfering with daily activities). The cut off for the severity score was at least moderate.

The frequency of the symptoms was graded 0-4; 0: never, 1: one day a month, 2: at least once per week, 3: at least several times per week, 4: every day. The cut-off for frequency of symptoms in PDS and EPS subgroups was determined in agreement with the Rome III criteria. FD patients were classified into PDS if they reported postprandial fullness and/or early satiation at least several times per week (frequency score of 3 or more). The EPS subgroup included those patients reporting epigastric pain at least once per week (score 2 or more). Those fulfilling both PDS and EPS criteria were classified as overlapping PDS-EPS group; the others were classified as “pure” PDS or “pure” EPS.

Data analysis

All FD patients were classified as PDS, EPS and overlapping PDS-EPS in accordance with the Rome III diagnostic criteria (1). The correlation between severity and frequency scores was explored by means of the Spearman correlation. The concordance of the symptom severity and frequency rating were evaluated by simple and weighted kappa statistics. To do so, for each symptom the frequency and the severity scores were distributed into four categories (0-3). Therefore, the severity scores were used in their 0-3 division as described above and frequency scores were transformed into a 0-3 range (0: never or one day a month; 3: every day). Thereafter, the percentage of concordance between the severity and frequency categories was analyzed. A kappa of 1 indicates perfect agreement, whereas a kappa of 0 indicates agreement equivalent to chance. A p-value of less than 0.05 was considered significant. Statistical analysis was performed using Excel and GraphPad Prism 5.

3.1.3. Results

Patient characteristics

Four hundred and twenty-one FD patients fulfilling the ROME III criteria (68% female, 41.6±0.8 years old and BMI: 22.3±0.22) were recruited for the study. The patients were subdivided in into “pure” PDS (34%, 70% females, 41.6±1.2 years old, BMI: 21.7±0.32), “pure” EPS (9%, 62% females, 45.5±3.0 years old, BMI: 23.5±0.91) and the overlapping PDS-EPS group (57%, 70% females, 41±1.1 years old, BMI: 22.6±0.34).

The most frequently reported symptoms in this patient cohort were postprandial fullness (82%) and bloating (78%) occurring several times per week with at least moderate severity in 75% and 72% respectively (see table 1). Epigastric pain was reported at least once a week by 66% of the FD patients and its severity was at least moderate in 56% of the patients. Early satiation and nausea were reported several times a week by 55% and 52% of the patients respectively, and their severity was at least moderate in 48% of both groups. Finally, the least frequent and severe symptoms were belching (frequency several times per week: 52% and with at least moderate severity: 39%) and vomiting (frequency several times per week: 19% and with at least moderate severity: 18%).

Table 1. The frequency and severity of FD symptoms. The severity of the symptoms was graded 0 (absent) to 3 (severe; interfering with daily activities). The cut off for the severity score of all symptoms was at least moderate. The frequency of the symptoms was graded 0 (never) to 4 (every day). The cut-off for the frequency of PDS symptoms and other symptoms was more than 3 (at least several times per week), and frequency of EPS symptoms was more than 2 (at least once a week).

		Frequency	Severity (at least moderate)
EPS symptoms **At least once per week**	Epigastric pain	66%	56%
	Epigastric burning	33%	47%
PDS symptoms **Several times per week**	Postprandial fullness	82%	75%
	Early satiation	55%	48%
Other GI symptoms **Several times per week**	Bloating	78%	72%
	Nausea	52%	48%
	Belching	52%	39%
	Vomiting	19%	18%

Symptom frequency and severity in EPS, PDS and overlapping group

All "pure" EPS patients reported epigastric pain at least once per week and 70% of these patients scored pain of at least moderate severity. In addition, 68% of the EPS patients reported epigastric burning occurring at least once per week and 62% of them scored this symptom to be at least moderate in severity. In agreement with the Rome criteria, postprandial fullness and early satiation never occurred at least several times per week in this group (Figure 1 A and B). Nevertheless, a small percentage of these patients reported postprandial fullness and early satiation with at least moderate severity once a week (14% and 3% respectively) and none reported these to occur once a month.

The highest symptom frequency and severity ratings in the PDS subgroup included postprandial fullness several times per week (90%, and 86% with at least moderate severity), bloating several times per week (77%, and 75% with at least moderate severity) and early satiation several times per week (61%, and 57% with at least moderate severity). In addition, nausea was also frequently reported (56%, 53% with at least moderate severity) (Figure 1 A and B).

For many symptoms, the highest frequency and severity scores were found in the overlapping PDS-EPS group. These included meal-related symptoms such as postprandial fullness several times per week (90%, and 80% with at least moderate severity), bloating several times per week (83%, and 74% with at least moderate severity), early satiation several times per week (59%, and 50% with at least moderate severity), and also meal-unrelated symptoms such as epigastric pain at least once per week (100%, and 87% with at least moderate severity) and epigastric burning at least once per week (56%, and 39% with at least moderate severity).

The frequency and severity of belching were similar in the three subgroups (frequency EPS: 51%, PDS: 48% and overlapping group: 54%; severity EPS: 30%, PDS: 39% and overlapping group: 41%). Finally, the frequency and severity of nausea were similar in overlap and in the PDS subgroup (51%, and 48% with at least moderate severity) (Figure 1 A and B).

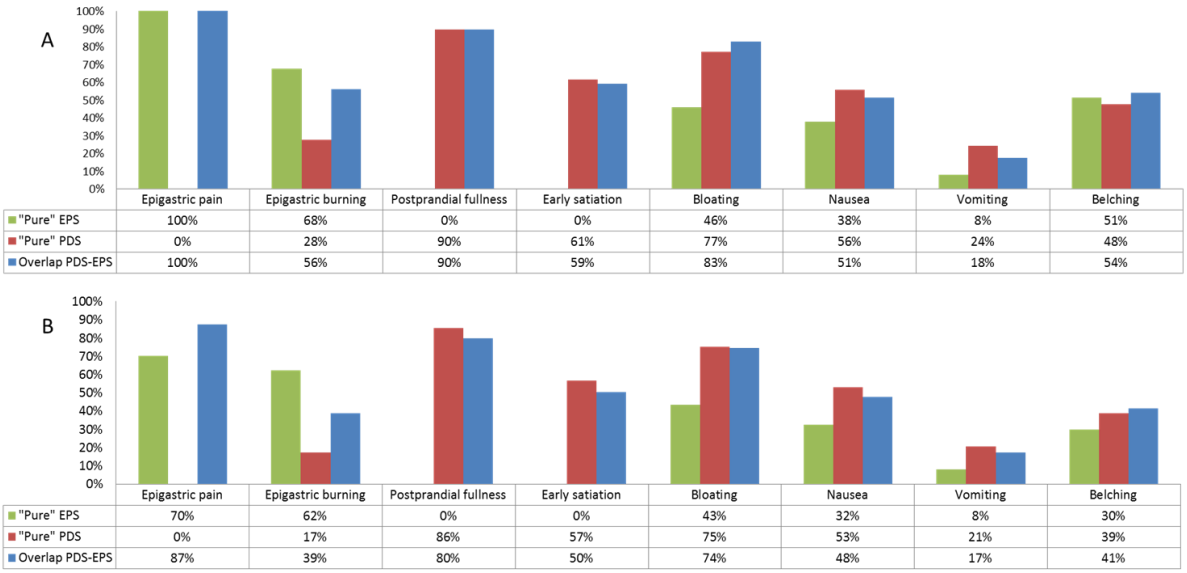

Figure 1. Symptom frequency and severity in FD subgroups. A. Frequency of symptoms. Patients were subdivided as per Rome criteria in "pure" PDS, "pure" EPS and the overlap EPS-PDS subgroups depending on the frequency of their symptoms. PDS was characterized by postprandial fullness and/or early satiation at least more times per week in the absence EPS symptoms. EPS was characterized by epigastric pain at least once per week in the absence of PDS symptoms. The overlapping EPS-PDS group comprised patients with both PDS and EPS symptoms. B. Severity of symptoms. The severity of the symptoms was graded 0 (absent) to 3 (severe; interfering with daily activities) in PDS, EPS and overlapping EPS-PDS subgroups. The cut off for the severity score was at least moderate.

Correlation of frequency and severity of symptoms

When the entire FD population was considered, positive correlations were observed between the severity scores and frequency scores for each symptom (Table 2).

Table 2. Spearman correlation analysis between frequency and severity of symptoms in all FD patients. *= p<0.05; **=p<0.01; ***=p<0.001

	Spearman r- value
Epigastric pain	0.79***
Epigastric burning	0.88***
Postprandial fullness	0.60***
Early satiety	0.86***
Bloating	0.72***
Nausea	0.87***
Vomiting	0.95***
Belching	0.88***

The frequency and the severity of most of the epigastric symptom were also positively correlated in all Rome III-defined subgroups. However, the frequency and severity of epigastric pain were least well

correlated in the EPS and in the overlapping group (EPS spearman r: 0.28, p=NS and overlapping group spearman r: 0.24, p<0.001) (Table 3). In addition, a relatively poor correlation was found for severity and frequency of postprandial fullness in the PDS and overlap groups (spearman r respectively 0.56 and 0.44).

Table 3. Spearman correlation analysis between frequency and severity of the symptoms in EPS, PDS and overlapping groups. *= p<0.05; **=p<0.01; ***=p<0.001

	EPS	PDS	Overlap EPS-PDS
Epigastric pain	0.28	0.79***	0.24***
Epigastric burning	0.78***	0.96***	0.81***
Postprandial fullness	0.90***	0.56***	0.44***
Early satiation	0.89***	0.78***	0.84***
Bloating	0.88***	0.80***	0.62***
Nausea	0.98***	0.87***	0.85***
Vomiting	0.99***	1.00***	0.94***
Belching	0.86***	0.96***	0.88***

The concordance between symptom occurrence and severity

In the entire patient cohort the classification of symptom severity and frequency showed moderate concordance for all symptoms (more than 60% of observed agreements). The frequency of vomiting showed the best concordance with its severity (83% of observed agreements) (Table 4).

Table 4. Number of observed agreements between frequency and severity scores in FD patients using Kappa analysis.

FD symptoms	KAPPA	SE	Agreement	Observed agreements
Epigastric pain	0.49	0.03	moderate	63%
Epigastric burning	0.54	0.03	moderate	69%
Postprandial fullness	0.44	0.04	moderate	63%
Early satiation	0.57	0.03	moderate	69%
Bloating	0.51	0.03	moderate	67%
Nausea	0.55	0.03	Moderate	68%
Vomiting	0.63	0.03	Good	83%
Belching	0.53	0.03	Moderate	67%

When subdividing FD symptoms, the frequency and severity of PDS symptoms showed a good concordance. In the PDS subgroup, there was little to no occurrence of epigastric pain, therefore showing poor concordance between its severity and frequency (Table 5).

The severity and frequency of most of the EPS symptoms showed a good concordance. However, the frequency of epigastric pain correlated poorly with the reported severity scores (Table 5).

In the overlap PDS-EPS group, only the occurrence of epigastric pain and postprandial fullness showed poor or fair concordance with the reported severity scores (see table 5).

Table 5. Number of observed agreements between frequency and severity scores in PDS, EPS and overlap EPS-PDS subgroups using Kappa analysis.

	FD symptoms	KAPPA	SE	Agreement	Observed agreements
PDS group	Epigastric pain	0	0	Poor	92%
	Epigastric burning	0.62	0.06	Good	81%
	Postprandial fullness	0.45	0.06	moderate	69%
	Early satiety	0.55	0.05	moderate	68%
	Bloating	0.65	0.05	Good	77%
	Nausea	0.66	0.05	Good	76%
	Vomiting	0.71	0.05	Good	87%
	Belching	0.6	0.05	moderate	72%
EPS group	Epigastric pain	0.12	0.10	Poor	49%
	Epigastric burning	0.57	0.10	moderate	68%
	Postprandial fullness	0.68	0.09	Good	84%
	Early satiety	0.74	0.10	Good	89%
	Bloating	0.56	0.09	moderate	68%
	Nausea	0.69	0.09	Good	81%
	Vomiting	0.60	0.11	moderate	92%
	Belching	0.43	0.08	moderate	59%
Overlap EPS-PDS group	Epigastric pain	0.14	0.05	Poor	50%
	Epigastric burning	0.47	0.04	moderate	62%
	Postprandial fullness	0.30	0.05	Fair	58%
	Early satiety	0.54	0.04	moderate	67%
	Bloating	0.41	0.04	moderate	60%
	Nausea	0.46	0.04	moderate	61%
	Vomiting	0.58	0.04	moderate	80%
	Belching	0.52	0.04	moderate	64%

3.1.4. Discussion

According to the Rome III criteria, mainly the frequency of symptoms is taken into account to establish the presence of FD, and to identify the subgroups of PDS and EPS (41, 42). The postprandial distress syndrome (PDS) subgroup is defined by the presence of postprandial fullness and/or early satiation after normal-sized meals at least several times per week. The epigastric pain syndrome (EPS) subgroup is characterized by epigastric pain and/or epigastric burning at least once per week (41, 42). In terms of severity, it has earlier been proposed to grade symptoms in chronic and functional disorders as mild, moderate or severe, according to their impact on the patient's daily functioning (208-210). Although functional dyspepsia is a benign disease without excess mortality (45), it is associated with a

major health economic and quality of life burden, and here both severity and frequency of symptoms may be important to consider (196, 209, 211).

In this study we explored the frequency and severity of symptoms in FD subgroups according to the Rome-III criteria. In the PDS subgroup, postprandial fullness and early satiation were the most frequent and severe symptoms, and their severities and frequencies were well correlated, indicating that a frequency assessment (e.g. through daily diaries), is able to provide a reliable estimate of their impact. The same was true for accessory symptoms such as upper abdominal bloating, belching and nausea. Previous studies have shown that PDS symptoms occur readily after meals (11), and hence, if they are triggered by every (major) meal, frequency for these symptoms may show limited variability.

In the EPS subgroup the most prevalent symptom was epigastric pain, but the severity and frequency for this symptom were poorly correlated. Hence, in EPS patients, severity and frequency of epigastric pain should probably both be assessed separately. The second most frequent and severe symptom in EPS was epigastric burning, and here frequency and severity correlated well. In the PDS/EPS overlap group, most of the symptoms showed a high frequency and a positive correlation with severity, with again an exception for epigastric pain. Also the concordance for postprandial fullness severity and frequency was lower in the overlap group.

The Rome questionnaires mainly serve for diagnostic categorization purposes. The most recently published Rome IV consensus does not assess severity, but assesses only frequency of symptoms that are more intense than the “bothersome” threshold severity (212). However, for quantification of symptom impact, severity may need to be taken into account, especially for the EPS symptoms. Specific questionnaires have been developed to measure the severity and quality of life impact of FD, and these also take into account severity ratings (211, 213-216). For instance, the PAGI-SYM questionnaire has been developed and validated for the evaluation of therapeutic responsiveness in upper gastrointestinal disorders, including FD (199, 214, 217). The NDI questionnaire addresses 42 items structured around 17 themes in order to gather information about disease-specific quality of life measure for dyspepsia (211). Both questionnaires have been used in clinical trials to assess symptom and quality of life responsiveness in FD patients. However, as both questionnaires use a 2-week recall, they are no longer in agreement with FDA guidelines for symptom evaluation (218). Daily diaries, as advocated by the FDA, provide some frequency assessment through their daily symptom evaluation, allowing to quantify the occurrence of symptoms expressed as proportion of days with symptoms, while severity is assessed in the daily severity rating. Following FDA guidance, we recently developed and validated the LPDS daily diary, a Patient Reported Outcome instrument for PDS. This diary also assesses severity ratings of PDS symptoms on a daily basis(219), using questions that were developed in focus group assessing the presence of symptoms (19). Based on the current analysis, development of a Patient Reported Outcome instrument for EPS or for overlapping patients would need to take into account both frequency and severity from the early stages.

In conclusion, assessment of PDS symptoms can be accomplished by measuring frequency or severity, as both are closely correlated. However, for the assessment of EPS symptoms, especially epigastric pain, both frequency and severity should be taken into account to assess symptom burden in FD.

3.2. Functional Dyspepsia: outcome of focus groups for the development of a questionnaire for symptom assessment in patients suffering from Postprandial Distress Syndrome (PDS)

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3.2.1. Introduction

Functional dyspepsia (FD) is one of the most prevalent functional gastrointestinal disorders, and is defined by Rome III consensus as the presence of epigastric symptoms in the absence of any organic or metabolic disease likely to explain these symptoms (41). From the symptom presentation and pathophysiological point of view, FD is a heterogeneous condition with different underlying pathophysiological mechanisms contributing to the symptom pattern (220). To facilitate diagnostic and therapeutic approach to FD patients the Rome III consensus proposed to subdivide FD into Postprandial Distress Syndrome (PDS), characterized by meal-related symptoms such as early satiation and postprandial fullness and, Epigastric Pain Syndrome (EPS) characterized by epigastric burning and epigastric pain (41).

Population and patient studies have shown that the largest subgroup of FD according to Rome III criteria is the PDS subgroup. It has been proposed that this is the patient group where prokinetics may offer potential symptom benefit (130, 134, 135). However, the evidence to support the efficacy of prokinetics in FD or PDS is limited, and meta-analyses of prokinetic therapy in FD are hampered by a dominant number of studies with cisapride, no longer available, and an apparent publication bias for older studies (221). Recent attempts to develop new prokinetics have been unsuccessful, probably for a variety of reasons including choice of drug and dose, patient selection and especially the use of inappropriate endpoints or endpoint questionnaires (119, 132, 222). A recent systematic review concluded that no validated tool for the evaluation of treatment responsiveness in FD patients according to the Rome III criteria is currently available (148).

Patient reported outcomes (PRO) questionnaires provide information on specific health concepts directly from the subjects without interpretation of the patient's response by a physician or others. The Food and Drug Administration (FDA) draft guidance from 2006 and final guidance, released in December 2009, provide recommendations for the use of validated instruments to assess treatment outcomes, and describes the proper development and psychometric validation of patient reported outcomes (PRO) questionnaires to be used in evaluation of new therapeutic agents (150). The Rome III committee has also provided guidelines for clinical trial design in FGIDs, with a similar emphasis on individual patient assessment and the use of validated outcome measures (223).To date, no PRO is available to evaluate symptom severity in FD in line with Rome III and FDA guidelines (149).

Our aim is to develop and validate a PRO questionnaire for PDS, based on the US FDA guidance for PROs. The choice to start with the PDS subgroup is driven by the larger proportion of PDS patients compared to EPS patients and by the availability of a large group of prokinetics that need to be studied in this patient group (130, 134, 135, 221, 222). In agreement with FDA guidelines, recorded structured interviews in patient focus groups were used to identify symptom items that are relevant to PDS patients.

3.2.2. Materials and Methods

Patient selection for focus groups

Consecutive ambulatory patients between 18 and 70 years with a main diagnosis of functional dyspepsia - postprandial distress syndrome (PDS), and no other overlapping major symptomatic conditions, were eligible to participate in this study. Patients referred for dyspeptic symptoms and a negative upper gastrointestinal endoscopy were selected using the ROME III FD questionnaire with some additional gastro-esophageal reflux disease (GERD) questions (194). Patients had to fulfill the ROME III criteria for PDS, implicating that they reported bothersome postprandial fullness or early satiation occurring after normal-sized meals at least several times per week during the last 6 months. Moreover, in addition to fulfilling the ROME III PDS criteria, patients were excluded if they had frequent and bothersome co-existent GERD symptoms, EPS symptoms, chronic idiopathic nausea, excessive belching and reflux. The selected PDS patients were invited to focus groups, aimed to comprise 5 to 8 patients and held at Leuven University Hospital, to address the nature and impact of their symptoms.

Focus Group session setup

Focus groups sessions were designed to identify relevant symptoms in PDS patients and to acquire detailed information about the nature and time course of these symptoms. Furthermore, during the sessions, possible instrument items such as statements and rating scales could already be evaluated for clarity (224).

A minimum of three focus group sessions were planned to be held, each including 5 to 8 PDS patients, and to be increased until saturation of identified symptom items was reached. The small number of patients per focus group was chosen to facilitate management of the dialogue and discussion (223, 224). However, this number is considered large enough to provide information on variations between groups (223, 224).

The focus group discussions were moderated by an experienced physician who used a specific framework as a guide. This framework was prepared to address all relevant symptoms in the Dyspepsia Symptom Severity Index (DSSI), but care was taken to leave plenty of room for additional open questions to facilitate discussion. The DSSI (developed under the Rome I and Rome II definitions) is the only FD questionnaire which is designed to allow distinguishing meal-related and meal-unrelated occurrence of several epigastric symptoms (148, 225). In line with epidemiological studies (19-26), in line with the DSSI framework (18), and in agreement with more recent patient- and expert-based evaluations of upper gastrointestinal symptom complexes (41, 74, 174, 181, 213, 226), upper gastrointestinal symptoms were conceptualized to fall into 4 domains, respectively referring to PDS, EPS, GERD and other disorders (which include nausea/vomiting and belching disorders) (Figure 1 and Table 1). All focus groups were attended by three additional investigators (clinicians and research assistants) who observed and took notes, and all focus group sessions were audio-taped for subsequent analysis.

Conduct of the Focus Group

After a welcome and an introduction about the nature of the project to the patients, the session started and was conducted in a specific order. First, general open questions were asked with the intention of determining the general experiences on epigastric symptoms. Patients were asked

whether they considered themselves to have stomach symptoms and to identify the part of the abdomen where these were mostly felt. Moreover, the relationship of these symptoms to meal ingestion was explored; participants were given abundant time to express themselves.

Next, open-ended questions were asked to address specific upper gastrointestinal symptoms, in line with the conceptual framework (Table 1). Besides the cardinal PDS symptoms of early satiation and postprandial fullness, additionally discussed items included upper abdominal bloating, epigastric pain and burning, nausea, heartburn and vomiting. After that, the patients were invited to mention and discuss any other relevant symptoms. For each symptom, patients were asked whether they experienced it and to elaborate on its characteristics, frequency of occurrence, relationship to meal intake, duration, impact and threshold frequency to be considered bothersome. Furthermore, they were also asked whether the frequency, consistency and intensity of the symptoms depended on the type of meal they ate and whether the severity of these symptoms could be decreased by any specific measures.

During the discussions different verbal descriptions of symptom items, based on the DSSI but also other questionnaires, were projected on a screen (148, 213, 225). The participants were invited to interpret the items out loud. They could paraphrase, define or comment the used terms in order to identify ambiguous or poorly worded questions, and to express preference for one of the wordings (227). Moreover, the participants were asked to express out loud their line of though in responding to each item, in order to obtain insight on the reflections leading to an answer (227). The information given by this discussion was later used to refine or improve the items in a draft questionnaire. Furthermore, patients could express preference for a type of rating: verbal descriptors (5 items ranging from absent to very severe) accompanied by numbers (1 to 5) or “smiley faces” (☺ to ☹).

Table 1. Items included in the conceptual framework. The numbers between brackets refer to the number of the question in the Dyspepsia Symptom Severity Index (DSSI).

PDS symptoms	Inability to finish normal-sized meals (DSSI Q5)
	Feeling full after meals (DSSI Q4)
Possible PDS symptoms	Upper abdominal bloating (DSSI Q3)
	Stomach distension (DSSI Q7)
	Nausea after meals (DSSI Q12)
	Epigastric pain after meals (DSSI Q8)
	Stomach discomfort, without pain, after meals (DSSI Q6)
EPS symptoms	Burning feeling in your stomach (DSSI Q19)
	Stomach pain before meals or when hungry (DSSIQ9)
	Stomach pain at night (DSSI Q10)
Belching, nausea, vomiting symptoms	Frequent burping or belching (DSSI Q1)
	Nausea before meals (DSSI Q11)
	Nausea when you wake up in the morning (DSSI Q13)
	Retching (DSSI Q14)
	Vomiting (DSSI Q15)

GERD symptoms	Burning feeling in your chest (heartburn) (DSSI Q18)
	Burping with bitter tasting fluid in throat (DSSI Q2)
	Regurgitation of bitter fluid into your mouth during the day (DSSI Q16)
	Regurgitation at night (DSSI Q17)

Cognitive interview

For this phase, also patients with a PDS diagnosis according to Rome III criteria were eligible, but co-morbidities like co-existing EPS, GERD or nausea, but no vomiting, were allowed. The symptom items identified during the focus group sessions were expressed as questions to be evaluated in a pilot PRO instrument by means of a 5 point-scale. Symptoms reported by 50% or more of the participants in the focus groups were addressed in-depth in the cognitive interviews, but questions dealing with severity of upper abdominal bloating and potentially overlapping EPS, nausea and belching disorders were also pre-planned to be included.

Cognitive interviews were conducted on a one-to-one basis by one of the investigators (FC). To evaluate the relevance, clarity and consistency of each of the symptom item questions, PDS patients provided a written response, with verbal discussion, to 4 questions regarding the symptom item description (227). The symptom item description used was the one with the majority preference from the focus groups. First, the comprehension of the item description was addressed by asking "In your own words, what is this question asking about? Are there any words in this question that you do not understand? What does the symptom mean to you?". Second, the decision-making process was addressed by asking "What do you think of the choice of answers? How did you select your answer?". Third, the adequacy of the recall period was addressed by asking "Are you able to accurately remember how bad this symptom was in the past 24 hours?". Finally, the three most preferred descriptions from the focus group interviews for the symptom were provided, and the participants were asked to express their preference for one of the three sentences, based on their personal interpretation and experiences. This was helpful to identify optimal wording and tone for patients in describing symptoms that they recognize as relevant (227).

Data analysis

Focus group data processing

Demographics and clinical information of the individual participants were obtained from the Rome III questionnaires, the endoscopy findings and the outpatient clinic documents, assessed during the patient selection process. After the group sessions, the moderator and note-takers summarized and reviewed the answers to the questions, discussion and observations into a final consensus report (223, 224). Focus groups were conducted to reach a minimum of 3, or until saturation of reported PDS symptoms was obtained. Saturation is achieved when the number and type of cardinal symptoms mentioned in the sessions is stable, and when the symptoms are established with an understandable meaning and importance to most of the patients (228).

Cognitive interview data processing

The analysis and interpretation of the cognitive interview was based on the study of Knafl et al (227). A spreadsheet database was constructed to summarize statements and to facilitate the comparison

between the different patient's interpretations and feedback of each item. Afterwards, the characteristics of possible problems and the quality of the interpretations of the items were used to evaluate whether to preserve, modify or revise the symptom item question.

3.2.3. Results

Focus Group participants

Consecutive ambulatory patients presenting with dyspeptic symptoms (n=229) filled out Rome III gastro-duodenal questionnaires and a word-picture questionnaires to diagnose gastro-esophageal reflux symptoms in FD. A total of 54 patients were diagnosed with FD. These patients had typical FD symptoms for at least the 6 months before diagnosis, with a negative endoscopy and no major overlapping GERD symptoms. Of these, 26 had PDS as single final diagnosis without co-existing major EPS, chronic nausea and excessive belching (Figure 2). All 26 patients were invited to one of the 3 planned focus group sessions. A total of 15 PDS patients (5 per focus group) were able to participate at one of the sessions. The majority (87%) were female, they had a mean age of 48±3.2 years (range: 26-65 years) and a mean body weight of 61±4.3 kg. The patients reported upper abdominal symptoms since 5±1.2 years (range: 1-15 years).

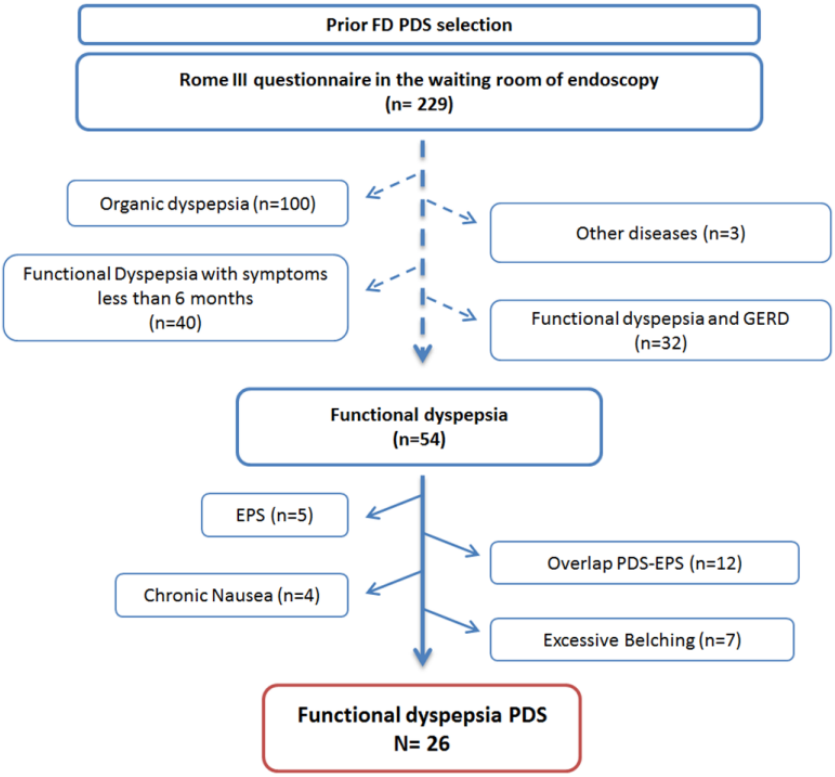

Figure 2. Selection of PDS patients. Consecutive ambulatory patients presenting with dyspeptic symptoms (n=229) filled out a Rome III gastro-duodenal questionnaire with additional questions to identify GERD. Those diagnosed with PDS FD (n=26) were invited to participate on the FG sessions.

Focus Group responses

All patients (100%) confirmed experiencing symptoms that were triggered or aggravated by ingestion of a meal. These symptoms corresponded to early satiation (100%) and postprandial fullness (100%) (Table 2). All patients reported a rapid onset of satiation, as a sensation of being excessively full

already occurring during or towards the end of a normal-size meal. In many instances, this feeling induced the end of food intake although many participants indicated habitual premature cessation of food intake to prevent this symptom from occurring.

Postprandial fullness was reported to start approximately 15 minutes after the meal and to easily persist for several hours after the meal. This feeling was described by the patients as a feeling of excessive heaviness in the stomach, as if being filled with bricks, and accompanied by a sense of stasis of food in the stomach for hours. In case of severe prolonged presence of the symptom, it could lead to the patient skipping the next meal.

Sensations of early satiation and postprandial fullness could be triggered both by solid food and by drinks, according to the patients. However, the type of food could determine the rapidity of onset of symptoms, as well as their intensity and duration. All patients reported some personalized dietary adjustments, in which they restrict the quantity but also the type of food ingested and the timing of the meals. Nevertheless, the overall impact of these dietary restrictions on symptom occurrence, intensity and duration was reportedly rather limited. All participants agreed that early satiation and postprandial fullness are bothersome symptoms, deserving medical attention if they occur at least once a week after a normal sized meal. Additionally reported gastroduodenal symptoms included nausea, reported by 40% of the patients. Of these, all patients reported postprandial nausea (40%) whereas only a smaller proportion reported interprandial nausea (20%). Other symptoms included upper abdominal bloating (33%), excessive belching (27%), spasm-like cramps in the stomach region (27%) and vomiting (13%). Epigastric pain and burning were present in respectively 20% and 13%. The open discussion revealed that the term nausea was interpreted in various ways by patients. A subset of the patients equaled nausea to a generalized malaise, rather than a desire to vomit or a phase preceding potential vomiting. After clarification of the meaning of nausea in the sense of a sick sensation that precedes the need to vomit, only a subgroup of these patients indicated to have frequent and bothersome nausea. In those patients reporting bothersome nausea, this symptom was mostly present after a meal and occurred more rarely between meals (Table 2).

Table 2. Outcomes of symptom itemizations in 3 focus groups for PDS patients

	Symptoms	**Focus Group 1**	**Focus Group 2**	**Focus Group 3**	**%**
PDS symptoms	Postprandial fullness	5	5	5	100%
	Early satiation	5	5	5	100%
EPS symptoms	Epigastric pain	2	1	0	20%
	Epigastric burning	2	0	0	13%
Additionally reported symptoms	Nausea	2	2	2	40%
	Postprandial Nausea	2	2	2	40%
	Interprandial Nausea	2	1	0	20%
	Vomiting	0	1	1	13%
	Upper abdominal bloating	2	0	3	33%
	Stomach spasms	1	2	1	27%
	Excessive belching	1	1	2	27%

Non-gastrointestinal symptoms	Heartburn/Pyrosis	3	2	0	33%
	Weight loss	5	5	4	93%
	General fatigue	4	3	3	67%

Upper abdominal bloating was also a topic with variable interpretations. To some patients, this was interpreted as being identical to postprandial fullness. In the Rome definitions, upper abdominal bloating is described as an unpleasant sensation of gaseous distension located in the epigastrium. After explanation of the location and especially the association with a sense of distention by gas or air, only a minority of participants reported this as a bothersome symptom and very few reported sometimes visible expansion of the abdomen.

Belching was an infrequent bothersome symptom, and some participants reported that belching could lead to a slight temporary improvement of PDS symptoms. Epigastric pain was infrequently reported and, if present, mostly occurred postprandial in this PDS patient cohort. Epigastric burning was an infrequent symptom.

Non-gastroduodenal symptoms that patients reported included heartburn (present in 33%, but mostly occasional and depending on the type of meal ingested), weight loss (93%), and fatigue (67%). The fatigue was clarified to be a sense of general malaise and weakness following a meal, and was considered a very bothersome symptom. Upon its occurrence, some patients indicated they preferred to sit down while others prefer to take a short walk; some patients indicated that lying down after the meal rather worsens than improves the sensation of fatigue. The majority of patients reported weight loss, in the range of 0-12 kg, with an average of 5.0±1.7 kg. Most patients reported preserved appetite (sense of hunger), but attributed weight loss to decreased intake of food due to significant early satiation and postprandial fullness symptoms.

Cognitive interviews

A total of 15 patients with a diagnosis of PDS were recruited for the cognitive interview. The majority (80%) were female and they had a mean age of 40.6 years (range: 19-70 years). All patients confirmed experiencing symptoms such as early satiation (60%) and postprandial fullness (87%). Additional bothersome symptoms included upper abdominal bloating (67%), epigastric pain (60%, but mostly occurring postprandial), epigastric burning (20%), nausea (40%) and belching (67%).

All patients (100%) confirmed that they were able to remember their symptoms and their impact during the past 24 hours. All (100%) participants indicated that they understood the terms and wording used in the proposed draft questions very well. In choosing severity rating responses, the majority (60%) of the patients indicated they used both the smiley faces and the associated severity wordings. The remaining 40% used mainly the words to select severity ratings. Finally, patients were asked to express their preference among three different wordings for questions to address the severity of postprandial fullness and early satiation. Two thirds of the patients expressed a preference for one of the wordings, and the preferred descriptor sentences were the same as those with the highest preference by the focus group participants (Table 3).

Table 3. Preferences for wordings for symptom items in cognitive interviews with PDS patients

Cognitive interview item multiple choice		Preference
Early satiation	A. Hoe erg was vandaag het gevoel van **vroegtijdig verzadiging**? *How bothersome was the sensation of **early satiation** today?*	13%
	B. Hoe erg was vandaag het gevoel te **snel moeten stoppen met eten**? *How bothersome was the sensation of **have to stop eating earlier** today?*	20%
	C. Hoeveel last had u vandaag om een normale maaltijd t**e beeindigen omdat u te snel vol zat**? *How bothersome to finish a normal sized meal because you were **full too fast?***	67%
Postprandial Fullness	A. Hoeveel last had u vandaag van een zwaar gevoel in de maag na de maaltijd? *How bothersome was the sensation of **heaviness in your stomach after the meal**?*	27%
	B. Hoeveel last had u vandaag van trage vertering? *How bothersome was the sensation of **slow digestion**?*	7%
	C. Hoe erg was voor u vandaag het gevoel dat uw eten op uw maag bleef liggen? *How bothersome was the sensation that your **meal remained in your stomach**?*	67%

3.2.4. Discussion

Functional dyspepsia is a highly prevalent gastrointestinal disorder with considerable individual and socio-economic impact (43, 44). To date, no validated instrument for the evaluation of treatment efficacy in FD is available (148), and this is probably one of the reasons for the paucity of drugs of proven efficacy for this condition. In the absence of a suitable biomarker, therapeutic efficacy in FD needs to be evaluated based on the assessment of the patient's symptom pattern, frequency and severity (149). In agreement with the 2009 FDA guideline, specific patient reported outcome (PRO) instruments should be developed as "a questionnaire based on patient's perspective information to measure treatment effect in medical drug clinical trial" (150).

The present study was conducted as a first step in evaluating a PRO instrument for FD - PDS. We used the Rome III questionnaire to identify consecutive patients with a negative endoscopy, without predominant GERD as assessed by a word-picture questionnaire, and with predominant PDS symptoms (41, 194). These patients were invited to participate in interactive focus group sessions, facilitated by an experienced physician, using a conceptual framework based on the DSSI questionnaire as well as numerous other symptom descriptions in the literature (43, 47, 99, 225, 229-231).

The symptom pattern reported by the focus group patients confirms that they suffered from PDS symptoms as defined in the Rome III consensus: all participants reported symptoms that were triggered or aggravated by meal ingestion, and these symptoms consistently corresponded to early satiation and postprandial fullness. Other gastroduodenal symptoms such as upper abdominal bloating, belching or nausea (after clarification of its association with the desire to vomit), were reported only by a subset of the PDS patients. When nausea or epigastric pain were reported, they were mainly reported to occur postprandial, and it has been suggested that these may in fact be true PDS symptoms (41, 232).The patients also reported a number of general symptoms like fatigue or weight loss. Fatigue has already been reported as an important symptom in gastroparesis, where it has a major negative impact on quality of life (233). In studies using ultrasound to measure postprandial antral diameter, the occurrence of drowsiness after meals was significantly related to the antral diameter (234, 235). The patients in the current cohort also indicated significant weight loss, which is usually considered an alarm symptom (235). In the current cohort, the patients attributed the weight loss to symptoms of early satiation and postprandial fullness. Previous studies, both in FD patients and in the general population have shown associations of unintentional weight loss with early satiation and postprandial fullness (22, 60, 230). On the other hand, symptoms such as weight loss and fatigue are also influenced by psychosocial factors such as depression and somatization levels (60, 236).

The focus groups identified the symptoms of early satiation and postprandial fullness as the only symptoms reported by more than 50% of PDS patients. These were addressed in-depth in the cognitive interviews, but questions dealing with severity of other potentially relevant symptoms such as upper abdominal bloating, epigastric pain, epigastric burning, nausea and belching were also included. Again, all PDS patients selected for this part of the study confirmed that they experienced symptoms such as early satiation and postprandial fullness. All participants indicated good comprehension of the terms and wording of the proposed items, good recall of the severity of these symptoms over the last 24 hours, and they were at ease with the use of the rating scale. The preferred descriptor sentences for the symptoms of early satiation and postprandial fullness were the same as those preferred by the focus group participants, which shows a consistency between both patient groups.

In conclusion, the focus group sessions in PDS patients confirmed that symptoms corresponding to postprandial fullness and early satiation are the key items for developing a PRO for PDS in line with Rome III and FDA guidelines. They identified question wordings and rating scales for these symptoms that were relevant and easy to understand and answer by PDS patients, as confirmed in cognitive interviews. Further validation of a PDS PRO in a treatment trial is needed to determine responsiveness of the questions and their rating.

3.3. Validation of the Leuven Postprandial Distress Scale (LPDS), a questionnaire for symptom assessment in Functional Dyspepsia - Postprandial Distress Syndrome

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3.3.1. Introduction

Functional dyspepsia (FD) is a common functional gastrointestinal disorder, occurring in up to 20% of the adult population in western countries (41-43). The Rome III consensus defined FD as "the presence of symptoms thought to originate from the gastroduodenal region in the absence of any structural or metabolic disease that is likely to explain these symptoms" (41, 42). Furthermore, to facilitate the diagnostic and therapeutic approach to FD, the Rome committee proposed to distinguish two subgroups, taking into account the main symptoms and their relationship to meals (41, 42). A smaller subgroup of FD patients is classified as having Epigastric Pain Syndrome (EPS) which is characterized by meal-unrelated symptoms such as epigastric burning and epigastric pain. A larger proportion is classified as suffering from Postprandial Distress Syndrome (PDS), characterized by meal-relate symptoms such as early satiation and postprandial fullness (42, 46). However, in clinical practice, overlap between PDS and EPS occurs in a large subset of patients (42, 53, 55, 169). In FD as a group, ingestion of a meal is the most important trigger for symptom occurrence (78, 169, 195), and we recently showed that adapting the Rome III subdivision by taking into account the meal relationship of FD symptoms reduces the overlap between PDS and EPS through an increase in those that can be classified as PDS (169, 237).

In spite of its high prevalence, there is a lack of treatments with established efficacy for PDS. It has been proposed that prokinetic agents are the most suitable approach to offer symptom benefit to PDS patients, but several recent attempts to develop new prokinetics have been unsuccessful (96, 119, 132, 222, 238, 239). This is probably attributable to a variety of reasons, including choice of drug and dose, patient selection and especially the use of inappropriate endpoints or questionnaires to assess them (240). At present, no validated instrument is available for the assessment of symptoms and their responsiveness to treatment in patients suffering from PDS (148-150). In line with regulatory guidelines for development of Patient-Reported Outcome (PRO) measures (19, 148, 150, 240), we have recently conducted focus group sessions in PDS patients as defined by Rome III criteria, to identify all relevant symptom items that characterize PDS. Using the Dyspepsia Symptom Severity Index (DSSI) as guidance, but taking care to leave plenty of room for additional symptoms or questions, we identified the predominant symptoms in this population, as well as a number of secondary or accessory symptoms (19). These sessions were used to generate item questions on the cardinal and accessory symptoms that were later evaluated for content validity and adapted for relevance, clarity and consistency of phrasing by means of cognitive interviews in PDS patients (23). These new question items, developed in Flemish Dutch language, were translated to English and French and transculturally adapted in order to avoid biases in interpretation (19). The next steps in developing a PRO are the assessment of its validity and sensitivity to change (148, 150). The aim of this study was to validate the Leuven Postprandial Distress Scale (LPDS), in line with regulatory guidance, through the assessment of its consistency, reliability and ability to detect change in the framework of a controlled treatment trial.

3.3.2. Materials and Methods

Study design

This study was designed as an additional analysis of a double-blind randomized, multicenter, placebo-controlled study in PDS patients receiving either itopride 100 mg or placebo three times daily. However, the treatment medication was irrelevant to this study and the blind was not broken during this evaluation of the measurement properties of the LPDS. Use of a treatment trial allows the evaluation of responsiveness to change of the new symptom evaluation instrument (LPDS) by deliberately inducing change in some patients. After selection according to Rome III criteria for PDS, patients entered the eligibility period. During these 2 eligibility weeks, patients filled out the draft LPDS daily diary. If eligible based on the symptom pattern and frequency (see below), patients were then randomized in a double-blind fashion into the 2 parallel treatment arms. During this period, all patients continued to fill out the LPDS daily diary and additionally the Overall Treatment Evaluation, Overall Symptom Severity, PAGI-SYM and SF-NDI questionnaires at the end of the run-in period and after 2, 4, 6 and 8 weeks of treatment. During this period, there were three outpatient clinic visits (visit 3, 4 and 6) and one telephone call (visit 5) (Figure 1).

At the end of the study, patients were offered the possibility to enter an open label period of 8 weeks (Itopride 100 mg three times daily), which was included purely for the benefit of the patients (Figure 1). Patients enter an eligibility period for 2 weeks. At visit 2, patients were randomized into two treatment parallel arms (itopride vs. placebo) and were followed up for 8 weeks. Finally, patients could enter an open label period of 8 weeks.

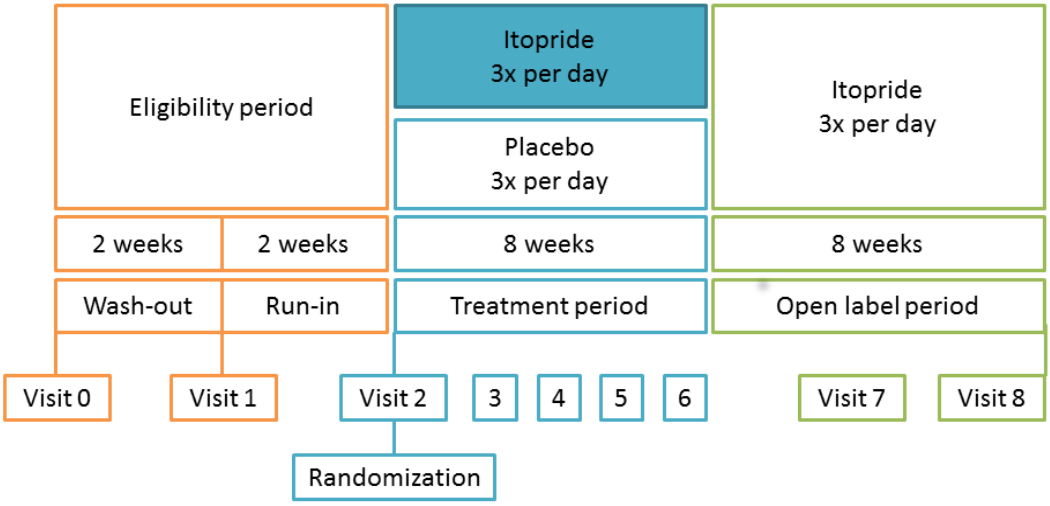


Figure 1. Design of the validation study of the Leuven Postprandial Distress Scale.

Patient selection

Consecutive outpatients diagnosed with PDS according to Rome III criteria at eleven gastroenterology practices in Belgium were eligible for the study. Both French and Dutch speaking patients between the ages of 18 and 70 years were included. FD patients were classified into the PDS subgroup, as in agreement with the Rome III criteria, if they reported bothersome postprandial fullness and/or early satiation occurring after normal-sized meals at least several times per week during the last 6 months. Following these criteria, patients were included if they were confirmed to suffer from active PDS as per LPDS scoring system during the 2 weeks eligibility period (see below). This required the presence of at

least moderate (score 2) postprandial fullness and/or early satiation symptoms on at least 4 days during the 2 weeks eligibility period.
All patients completed a previously validated gastro-esophageal reflux disease (GERD) questionnaire (194). Patients were excluded if they reported frequent and bothersome co-existent GERD symptoms, or if they had a history of reflux esophagitis. Furthermore, patients were excluded if they reported predominant symptoms of irritable bowel syndrome (IBS), chronic nausea (present every day), vomiting (more than once a month), excessive belching (present every day) and when patients presented symptoms of EPS several times a week according to Rome III questionnaire.
In addition, patients were excluded if they failed to fill out the questionnaires adequately. Female patients who were pregnant or lactating were also not eligible. Furthermore, patients were excluded if they had abnormal findings on upper gastrointestinal endoscopy, and if they had a history of upper digestive surgery, diabetes, coeliac disease, inflammatory bowel disease or any other disorder affecting upper GI motility such as dysphagia. Patients who were *H. Pylori* positive or patients who received treatment for HP eradication during the preceding 3 months were also excluded. Patients taking prohibited medication needed a wash-out period of 2 weeks prior to screening. Prohibited medications included those medications that could influence gastric physiology, motility and sensitivity or that could induce lesions in gut mucosa. Patients with an active psychiatric condition (major depression, anxiety disorder, alcohol or substance abuse) were excluded. However, patients who were taking a stable dose of a single antidepressant (except amitriptyline which was shown effective in FD (129) during the last 3 months were eligible.

Study drug

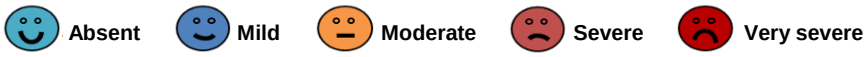
Itopride is a prokinetic benzamine derivative with dopamine-2 antagonistic and cholinesterase inhibitory properties, which generates a prokinetic effect on gastric motility (129-132, 222). Moreover, due to its high polarity it does not cross the blood-brain barrier, it does not induce clinically relevant increases in prolactin levels and it does not prolong the Q-T interval, making it a very safe compound. Itopride (100 mg three times daily) was chosen for this trial for its potential to improve symptoms (based on phase 2 and 3 studies in Europe), its attractive safety and tolerability profile (on the market in Asia since 1986 and studies conducted in Europe with up to 200 mg three times daily without major safety concerns), and its limited presence in the European market, allowing this study to offer a novel treatment approach to the patients (129-132, 222).

Questionnaires

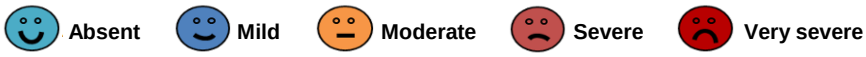
Symptom severity was assessed with a daily diary and with questionnaires that were completed at fixed time points during the trial. The questionnaires used were the Leuven Postprandial Distress Scale (LPDS) diary, Overall Symptom Severity assessment, Overall Treatment Evaluation, the patient assessment of upper gastrointestinal symptom severity index (PAGI-SYM) and the short form-Nepean Dyspepsia Questionnaire (NDI) (214, 241). Based on a literature survey, the Rome III definitions, focus group sessions and cognitive debriefing, a provisional diary instrument was constructed which consisted of 8 upper abdominal symptoms (early satiation, postprandial fullness, upper abdominal bloating, epigastric pain, epigastric burning, nausea, belching and heartburn) (19). The rating of the items is expressed as verbal descriptors (5 levels per item, ranging from absent to very severe) accompanied by “smiley faces” (☺ to ☹) (Figure 2) .

PDS symptoms

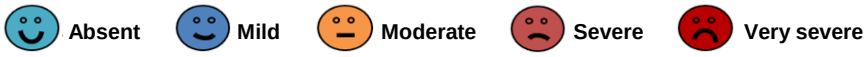
1. How hard was it for you to finish a normal meal today because you felt full too quickly?



2. How bad was the feeling today that your food was lying heavily in your stomach?

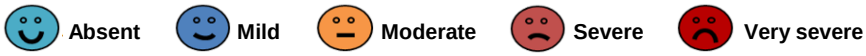


3. How much did you suffer from feeling bloated in your stomach today?

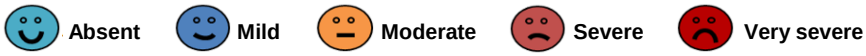


Accessory symptoms

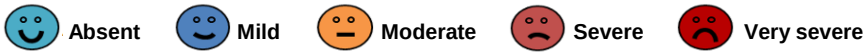
4. How much did you suffer from pain in your stomach area today?



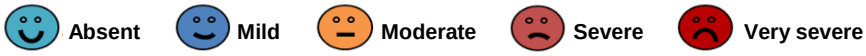
5. How much did you suffer from a burning feeling in your stomach area today?



6. How much did you suffer from nausea (feeling sick) today?



7. How much did you suffer from troublesome burping today?



8. How much did you suffer from a burning feeling behind your breastbone (in your chest) today?

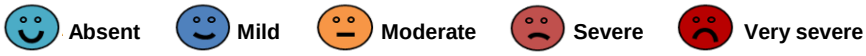


Figure 2. LPDS Questionnaire

Weekly change of the symptoms was assessed by Overall Treatment Evaluation and Overall Symptom Severity scales. In the Overall Treatment Evaluation question, patients were asked to indicate on a 9-point scale if their dyspepsia symptoms had improved, remained unchanged or had worsened since the last evaluation before starting the treatment. This is a very commonly used global endpoint but it may be biased due to the length of the recall period prior to the treatment (213, 242, 243). In the

Overall Symptom Severity question, patients indicate the overall severity of their dyspepsia symptoms in the last week on a 6-point likert scale (0, no symptoms present; 5, very severe symptoms) every 2 weeks. In contrast to the Overall Treatment Evaluation question, this endpoint is not dependent on the recall period prior to study drug administration (213, 242, 244, 245). The Overall Treatment Evaluation and Overall Symptom Severity questionnaires have previously been used in different clinical and instrument validating studies (136, 213). As there is no gold standard for establishment of an MCID in these questionnaires, there is widespread agreement in which one-point change of the Likert-type scales is defined as the MCID. The Overall Treatment Evaluation, in particular, has descriptors that indicate a qualitatively distinct change in symptom state for every point on the scale.

The PAGI-SYM has been developed and validated for the evaluation of therapeutic responsiveness in FD (199, 214, 217). It consists of 20 items divided into 6 symptom subscales (heartburn/regurgitation, fullness/early satiety, nausea/vomiting, bloating, upper abdominal pain, and lower abdominal pain). Each item covers a range from 0 (none or absent) to 5 (very severe) and assesses symptom severity over the preceding 2 weeks; the subscales are obtained by averaging symptom group scores, also ranging from 0 to 5. It has previously been shown that the PAGI-SYM questionnaire has a good internal consistency and test-retest reliability, and there is evidence supporting its content and construct validity. Moreover, it has been translated in many languages and culturally adapted (199, 214, 217).The PAGI-SYM MCID values of fullness/satiation and upper abdominal bloating subscales were used in this study as guidance. The MCIDs for fullness/satiation and for bloating have previously been published as 0.87 and 0.90 respectively (242). The NDI questionnaire is a disease-specific quality of life measure for dyspepsia. It addresses 10 items distributed over 5 domains (tension/sleep, interference, eating/drinking, knowledge/control, and work/study) to assess the impact of FD on the subject's quality of life. All items are measured by 5-point Likert scales from 0 (not at all or not applicable) to 4 (extremely) (211, 215, 216).

Statistical analysis

The LPDS daily diary uses “smiley faces” (☺ to ☹) to score symptoms on an absent to very severe range. These were numerically transformed into a 0-4 score range, and averaged for each symptom as a weekly score (0-4). Use of a daily diary has the advantages of prospective recording rather than relying on potentially faulty recall (246) and averaging over a one week period yields more reliable measurements. Evaluation of the LPDS measurement properties utilizes baseline measures (visit 2 of the trial) which was considered the most reliable visit to measure symptom pattern and severity, as this is at the end of an evaluation period off potentially interfering pre-existing therapies, and prior to the start of placebo-controlled treatment. The end of treatment measurements (visit 6 of the trial) were used for the purpose of responsiveness. The LPDS is regarded as the test measure while a number of other instruments are used as external anchors (Overall Treatment Evaluation, Overall Symptom Severity, PAGI-SYM, NDI) in evaluating a number of aspects of the validity and reliability of measurement of the LPDS, as described below.

Based on focus group sessions in 15 PDS patients, early satiation and postprandial fullness were confirmed as cardinal PDS symptoms, to be included in the LPDS diary score. The inclusion of 6 accessory symptoms (upper abdominal bloating, epigastric pain, epigastric burning, nausea, belching and heartburn) in the provisional diary (19) was aimed at 1) detecting newly emerging PDS-associated symptoms by observing the prevalence of these symptoms in the recruited PDS population and by

construct validity statistical analysis (see below) and 2) allowing to make observations of potential treatment effects (improvement as well as worsening) on overlapping or accompanying symptoms when using the diary in a clinical trial.

Construct validity

The construct validity of the LPDS was assessed by means of confirmatory factor analysis which specifies the domain structure of the LPDS in a latent variable model in which each domain of the instrument is represented by a latent variable and each of the symptom items are observed variables at study visit 2. If the a priori specified model reproduces the observed correlations between symptom items well this is taken as support for the construct validity of the instrument. Confirmatory factor analysis model fit targets have been adopted from *Schermelleh-Engel et al.* (247) including the Chi-Square measure (ideally non-statistically significant), the ratio of Chi-Square to degrees-of-freedom (ideally <5), Goodness-of-fit (ideally >0.95), Comparative Fit Index (ideally >0.95) and Root Mean Square Error of Approximation (ideally <0.05). In addition, for the measurement model to support the hypothesized constructs all individual item loadings need to be statistically significant and consistent, although some variation in loading magnitude would not be surprising or harmful to the hypothesis.

Based on the Rome criteria LPDS items could be subdivided into two distinct constructs: 1) postprandial distress syndrome (PDS) items: early satiation and postprandial fullness, and 2) epigastric pain syndrome (EPS) items: epigastric pain and epigastric burning (2). Besides the cardinal symptoms of early satiation and postprandial fullness, the Rome III consensus also mentions upper abdominal bloating, nausea and excessive belching as potentially accompanying symptoms, and these were explored for inclusion in the instrument. Based on its high prevalence in the PDS patients (see below), upper abdominal bloating was also included in the PDS construct as a potential emerging symptom. Hence latent variables representing PDS and EPS were specified with observed symptom items loading, a priori, as described. No cross-loadings were allowed as these are hypothesized to be distinct symptom constructs.

Known groups (criterion) validity

Criterion validity was assessed by comparing groups which would theoretically be expected to differ in distribution of LPDS domain scores. For this purpose individuals scoring high (4-5) or low (1-3) on the Overall Symptom Severity scale were compared with respect to PDS and EPS scores using the Mann-Whitney test. A standardized measure of effect size, Cohen's d, is also reported to quantify the degree of differentiation between groups. Values of d>0.8 are generally considered 'large'.

Convergent validity

The convergent validity concept adds credibility to the LPDS by showing that it is related to other measures of dyspepsia symptom burden. The LPDS at visit 2 was correlated with the Overall Symptom Severity, with the NDI-SF and with the PAGI-SYM domains corresponding to early satiation and/or postprandial fullness, upper abdominal pain and lower abdominal pain. All NDI domains are potentially relevant but it was a priori hypothesized that the eat/drink domain of the NDI should yield the strongest correlation with the PDS domain of the LPDS. The LPDS would be supported as a new measure of dyspepsia burden if there are moderate, positive correlations with the other measures. If

the correlations are too high (>0.9) the LPDS would not be adding new information over and above existing measures.

Internal consistency

The extent to which items within each LPDS symptom domain were related was assessed via the measurement model on the visit 2 measurements. A more direct assessment was also undertaken through measures of internal consistency. Descriptively the correlations between items were calculated and these are expected to be positive and high (>0.7). Cronbach's α was also calculated although this may not be as high as typically desired due to the small number of items per construct (248, 249) Generally α values>0.6 or ideally>0.8 are sought.

Test-retest reliability

The LPDS was recorded at several times during the trial but essentially steady state is ideal for assessing test-retest reliability. For this purpose, visits 1 and 2 were used to assess test-retest reliability since no treatment was applied prior to visit 2. Two statistics were calculated, 1) the Wilcoxon Signed Ranks test to evaluate the within subject change in LPDS score and 2) the correlation between visit 1 and visit 2 scores. The within-subject change establishes the extent to which the mean score changes over time while the correlation establishes whether the relative order of scores remains constant even if the actual values change over time.

Minimum Clinically Important Difference (MCID) for the PDS domain of the LPDS

The aim of the MCID calculation is to determine how large the change in PDS score needs to be to have clinical value (246, 250). The adopted approach was consistent with the convergent validity approach previously described in which the external measures, Overall Symptom Severity, Overall Treatment Evaluation and PAGI-SYM (early satiety/postprandial fullness), were used to define the term clinically meaningful as a change of one point in the external measure. For likert-type scales this has been established as meaningful, while for the PAGI-SYM MCIDs have been established at 0.87 for the fullness/satiation and 0.90 for the Upper Abdominal Bloating domains (217), which have been rounded as 1.0 for the purpose of this study. The MCID was determined for each anchor at both visits 4 and 6 by regressing the change between visit 2 and visit 4 or 6 in the PDS domain of the LPDS (dependent variable) on the corresponding change in an anchor variable (independent variable). The slope of this regression model defines the MCID since it estimates how much the PDS changes within patients, on average, per unit (one point) change in the external measure. Scatterplots of dependent and independent variables were examined to check that there is no clear departure from the assumption of linearity. A MCID was not established for the EPS domain of the LPDS since the validation study inclusion criteria only pertain to items on the PDS domain and this is likely to result in generally low values for the EPS scores.

Missing data handling method

Since the LPDS is calculated over a window of seven days and these days are replicate observations, the value for an LPDS item was taken as the average of the days available as long as at least half of days in that window are available.

3.3.3. Results

Study population

Between January 2013 and October 2014, 91 patients were identified and signed the informed consent form. After eligibility evaluation, 60 PDS patients (83% females, 38.2±2.1 years old, 61.6±12.8 g and 1.66±0.09 m) were randomized. Of these, 55 completed the entire study and 5 patients dropped out of the study in the last weeks (week 6 or week 7), in general because of lack of efficacy of the treatment.

Rome III symptom frequency

The symptom grouping was as follows: all patients suffered from PDS (postprandial fullness (98%) and/or early satiation (78%) several times per week) according to the Rome III classification. Upper abdominal bloating at least several times per week was equally prevalent, as it was reported by 78% of the patients. Nausea occurring at least several times per week was present in 31% of the patients. Non-predominant and not bothersome EPS symptoms were allowed during the study and, consequently, EPS symptoms such as epigastric pain several times a week, were reported by 16% of the patients. Only 7% (n=4) of these patients experienced epigastric pain every day but this occurred mainly after meals. None of the patients scored positive for predominant reflux and 31% of the patients reported belching several times per week.

When subdividing the FD patients in agreement with the Rome criteria, 48% of the patients (n=29) were defined as “pure” PDS (several times per week and no EPS symptoms at all) and 52% (n=31) were defined as patients overlapping PDS and EPS (PDS patients with EPS symptoms at least once a week). However, in all patients, PDS symptoms were the dominant symptom in terms of frequency score and in the overlap group, epigastric pain was mostly meal-related based on the Rome III questionnaire (n=26 meal related, and n=5 not meal related) (Figure 3).

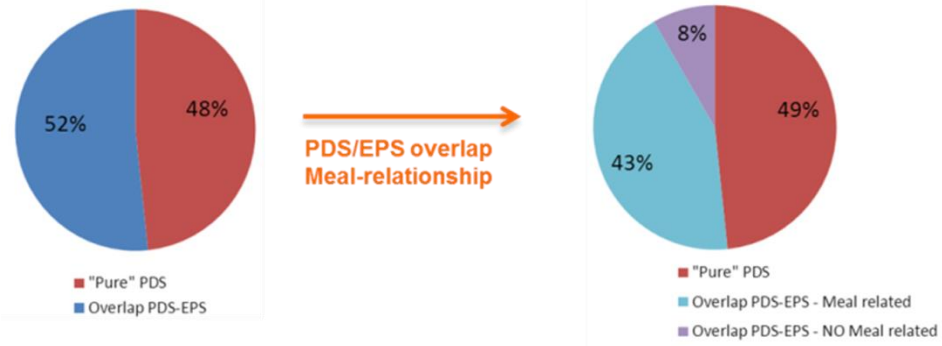


Figure 3. PDS and PDS/EPS overlap groups in the validation study of the Leuven Postprandial Distress Scale. The graph on the left depicts the proportion of the 60 enrolled patients who fulfill Rome III criteria for pure PDS and those who fulfill both PDS and EPS criteria. The graph on the right illustrates that the co-existing EPS symptoms are related to meal ingestion in the vast majority of patients.

Questionnaire symptom severity at baseline

The results of the LPDS diaries during the 2-week eligibility period and the PAGISYM scores at baseline are summarized in Supplementary Table 1. Diary scores for postprandial fullness, early satiation as well as upper abdominal bloating were high (mean ranging from 2.1 to 2.4). Intermediate scores were obtained for epigastric pain, nausea and belching (mean ranging from 0.9 to 1.2). Finally, patients

reported low severity scores for epigastric burning and heartburn (mean severity scores below 0.5). Similar results were obtained for the baseline symptom severity levels as assessed by the PAGI-SYM questionnaire.

Table 1. Mean severity score of LPDS and PAGI-SYM symptom scores at the end of the 2-weeks eligibility period in 60 patients recruited for the validation study of the Leuven Postprandial Distress Scale

	Early satiation	Postprandial fullness	Upper abdominal bloating	Nausea	Epigastric pain	Heartburn
LPDS (score 0-4)	2.1±0.1	2.4±0.1	2.1±0.1	1.0±0.1	1.2±0.1	0.4±0.1
PAGI-SYM (score 0-5)	3.4±0.2	3.8±0.2	3.0±0.2	1.9±0.2	1.8±0.2	1.4±0.2

Diary compliance

The percentage compliance with the LPDS diaries in the preceding period was assessed during the entire study at the end of every visit. Overall the daily diary average compliance (number of questions answered per day) was 91%.

Adverse events

Adverse events were reported by 67% of the patients of which 35% were recognized as adverse reactions (events with possible relationship with the drug). The most common possible adverse reactions to the study drug (itopride or placebo, as the code was not broken) were headache (13%), insomnia (5%) and dizziness (3%). No serious adverse reactions were reported.

Latent variable structure

Based on its high prevalence and symptom severity (Table 1), and in line with the Rome III concept (2), upper abdominal bloating was included with the 2 cardinal PDS symptoms. The two latent variable model hypothesized a priori in which early satiation (0.73±0.07), postprandial fullness (0.93±0.04) and upper abdominal bloating 0.83±0.05) form one construct and epigastric pain (0.93±0.13) and epigastric burning (0.60±0.12) form a second construct provided adequate fit to the data with a clear pattern of measures of model fit in the adequate range (Chi-Square $p=0.8$, Chi-Square/degrees-of-freedom=0.39, GFI=0.99, CFI=1.00, Root Mean Square Error of Approximation<0.01) (42). Further, all items yielded statistically significant ($p<0.001$) loadings on their respective latent variables (Figure 4). The standardized loading for epigastric burning on the second latent variable was numerically smaller than the others.

From these findings it can be concluded that the two-construct measurement instrument provides an adequate representation of the symptoms of FD/PDS according to the criteria used. It is further concluded that given the relatively homogeneous standardized loadings reported it is appropriate for uniform weights (1.0) to be applied to all items when calculating domain scores.

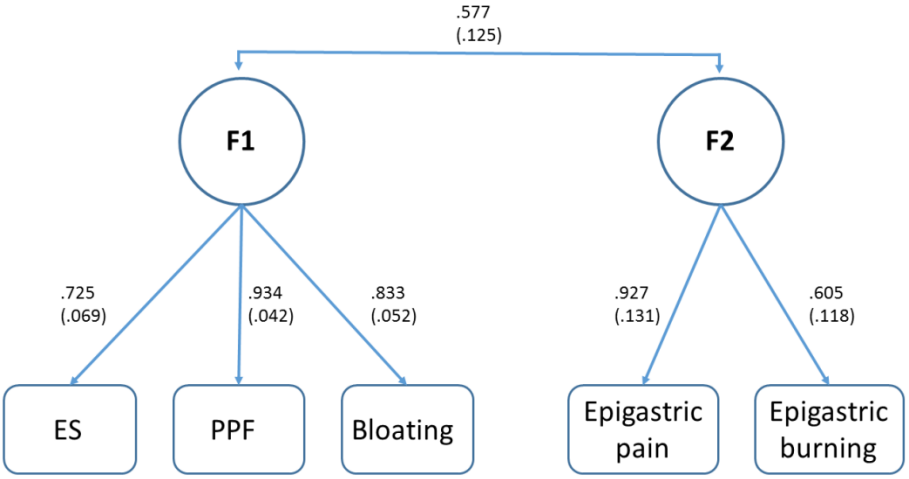


Figure 3. Confirmatory factor analysis model graphical representations. Data are based on 60 functional dyspepsia patients enrolled in the validation study of the Leuven Postprandial Distress Scale. The confirmatory factor analysis model of symptom ratings at Visit 2 (end of run-in) is displayed in graphical form

Known groups (criterion) validity

Both PDS and EPS mean scores were considerably and statistically significantly elevated in patients whose Overall Symptom Severity score was in the high range (4-5) compared with those whose Overall Symptom Severity score was not high (1-3). Cohen's d effect sizes were in the large range (>0.8) for both PDS (d=2.16) and EPS (d=1.24) (table 2).

Table 2. Known groups (criterion) validity (visit 2) in 60 patients recruited for the validation study of the Leuven Postprandial Distress Scale (LPDS). Know groups are patients with a low or high score on the Overall Symptom Severity scale (range 1-5). Scores for the PDS and the EPS symptoms in the LPDS diary are given as mean (SEM); p-values are based on the Kruskal-Wallis test. Cohen's d is a standardized measure of effect size. Values >0.8 are generally interpreted as 'large'.

Measure	Low OSS (1-3)	High OSS (4-5)	p-value	Cohen's d
PDS	4.7 (1.7)	8.8 (2.1)	<0.0001	2.16
EPS	0.7 (1.1)	2.5 (1.8)	<0.0001	1.24

Convergent validity

Cross-sectional correlations at visit 2 between the PDS and EPS scores on the one hand and Overall Symptom Severity, PAGI-SYM and NDI on the other demonstrated an appropriately differentiated pattern of positive correlations. OSS correlated strongly with both LPDS domains although more strongly with PDS than EPS. (Table3). The early satiation/postprandial fullness subscale of the PAGI-SYM correlated strongly with the PDS score, and less so with EPS but the reverse was true for the PAGI-SYM upper abdominal pain subscale. Of all NDI subscales, the eat/drink subscale was the most strongly correlated with both PDS and EPS (Table 2). The correlation of the Overall Treatment Evaluation with the longitudinal changes from visit 2 to visit 6 in PDS (r=-0.52, p<0.0001) and EPS (r=-0.02, p=0.872) suggested that changes in PDS domain, but not the EPS domain, correlated with patient

perceptions of outcome. From this appropriate pattern of correlations, it is concluded that the convergent validity of the LPDS is supported.

Table3. Correlation analyses between LPDS and other questionnaires. Cross sectional (visit 2) correlations are reported as Pearson correlation coefficients. Analysis was replicated using non-parametric Spearman correlation with no substantive difference in magnitude.

	PDS domain	EPS domain
Overall Symptom Severity	0.70 <.0001 52	0.48 0.0003 52
PAGI: PPF/ES	0.66 <.0001 55	0.48 0.0002 55
PAGI: Upper AP	0.30 0.02 55	0.64 <.0001 55
PAGI: Lower AP	0.05 0.74 55	0.49 0.0002 55
NDI: Tension	0.26 0.06 55	0.41 0.002 55
NDI: Interference (ADL)	0.18 0.18 55	0.13 0.33 55
NDI: eat/drink	0.58 <.0001 54	0.42 0.002 54
NDI: knowledge/control	0.17 0.21 55	0.21 0.13 55
NDI: Work/study	0.18 0.18 55	0.06 0.68 55

Internal consistency

Cronbach's α was high for PDS (0.86 at visit 2) and all inter-item correlations were high (0.67-0.76). α was moderate for EPS (0.72 at visit 2), as was the inter-item correlation (0.56 at visit 2) (Table 4). In calculation of the PDS score any one item can be omitted without undue loss of reliability; omitting bloating yields α=0.81, omitting post prandial fullness yields α=0.73 and omitting early satiety yields α=078.

Table4. Internal consistency in PDS and EPS domain.

	PDS domain			EPS domain		
Visit	**ES**	**PPF**	**Bloating**	**Epigastric pain**	**Epigastric burning**	**Chronbach's α**
2	1	0.68 <0.0001	0.578 <0.0001	1	0.56063 <0.0001	
2		1	0.78 <0.0001		1	
PDS	**0.66[A]**	**0.82[A]**	**0.73[A]**			**α=0.86**
EPS				**0.56[A]**	**0.56[A]**	**α=0.72**

[A]Correlations with full scale

α is Chronbach's α and is reported in standardized form. Table cell entries are Pearson correlations and p-value

Test-retest reliability

Test-retest reliability was evaluated in two steps. First, the within-patient changes in PDS and EPS scores between visits 1 and 2 were evaluated and found to be close to zero on average (mean change: PDS=0.02±1.47, EPS=0.09±0.76). Second, the correlation between visit 1 and visit 2 scores was evaluated (PDS r=0.85, EPS r=0.86, p<0.0001) (Figure 5). From these findings it can be concluded that the PDS and EPS scores of the LPDS are reproducible between two time points two weeks apart with no systematic change in patient management.

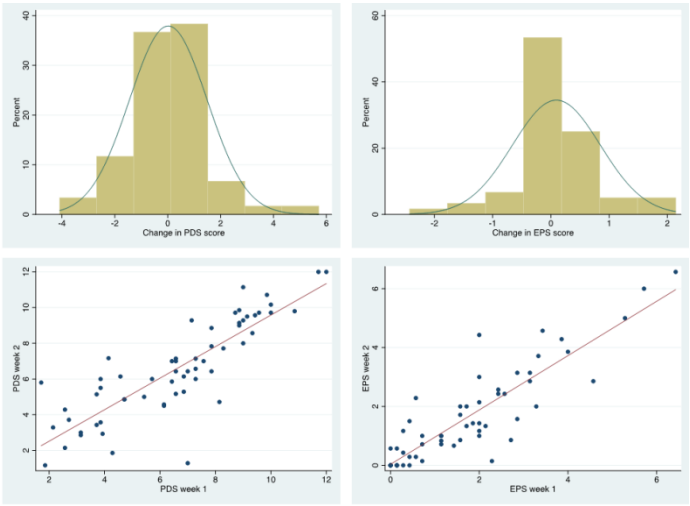


Figure 4. Upper panels: Graphical descriptions of change in scores between visits 1 and 2 in 60 functional dyspepsia patients enrolled in the validation study of the Leuven Postprandial Distress Scale. Pds1-pds2 pertains to PDS while eps1-eps2 pertains to EPS. Lower panels: Scatterplots correspond to the correlations between scores at both visits for PDS and for EPS respectively.

Minimum Clinically Important Difference (MCID) for the PDS domain of the LPDS

Estimated MCID values were determined to be consistent between 6 within each anchor variable but to vary between 0.4 and 0.6 depending on the anchor. An MCID of 0.5 would therefore correspond to a change of approximately one point in any anchor considered and hence would be considered

clinically meaningful. The results showed significant clinical meaningful change with all anchors: Overall Symptom Severity (MCID: 0.58±0.11), PAGI-SYM (MCID: 0.70±0.12) and Overall Treatment Evaluation (MCID: -0.49±0.12).

3.3.4. Discussion

To date, no validated PRO instrument is available for the evaluation of symptom severity or treatment efficacy in PDS patients. This is probably one of the reasons for the paucity of drugs of proven efficacy for this condition. We recently started to develop a new outcome assessment tool for PDS, in agreement with published experience and guidance for the development of Patient-Reported Outcome measures (19, 148, 150). From the results of the focus groups and cognitive interview we created a draft diary which included the cardinal PDS symptoms (postprandial fullness and early satiation) and several accessory upper gastrointestinal symptoms such as upper abdominal bloating, nausea and EPS symptoms that often co-exist with PDS (19). The individual symptoms are rated on a 0-4 severity scale with verbal descriptors and emoticons. This report describes the validation of this Patient- Reported Outcome tool in a PDS patient population through evaluation of its validity, reproducibility and sensitivity to change in the framework of a controlled trial. For this study 60 evaluable PDS patients were randomized after a 2-week run-in period in an 8-week double-blind placebo-controlled trial of itopride 100 mg three times daily They filled out the LPDS questionnaire as a daily diary and additionally filled out the Overall Treatment Evaluation, Overall Symptom Severity , PAGI-SYM and NDI questionnaires at 2-week intervals.

The diary questions that were evaluated were developed based on focus groups and cognitive debriefings in FD/PDS patients (19). Symptom prevalence profiles and factor analysis confirmed that the principal latent variable is comprised of the PDS symptoms of postprandial fullness, early satiation. In addition, based on its high prevalence and closer correlation with the cardinal symptoms, upper abdominal bloating was also included in the instrument. Moreover, inclusion of this symptom adds information that is not covered by the early satiation and postprandial fullness questions. The non-predominant EPS symptoms, present in only half of the patients corresponded to the second latent variable.

Using the mean diary ratings of the 3 PDS symptoms emerging from the symptom pattern analysis (postprandial fullness, early satiation, upper abdominal bloating) generated an LPDS score which correlated well with known-severity groups, based on the patients' OSS ratings. Convergent validity of the instrument was demonstrated based on its correlation with OSS ratings and with scores in the early satiation/postprandial fullness domain of the PAGI-SYM and the SF-NDI eat/drink dimension. Using the first and second visit, both preceding the start of treatment in this controlled trial; the internal consistency and reproducibility of the LPDS score were confirmed.

Changes in the LPDS correlated well with patients' Overall Treatment Evaluation ratings during the study. We used these, as well as the changes in Overall Symptom

Severity rating and in the PAGI-SYM domains of early satiation/postprandial fullness and upper abdominal bloating as an anchor to determine the MCID in LPDS score. These analyses converged on an MCID of 0.5 on the 0-4 range of the LPDS.

In the setting of a clinical trial, the LPDS average score can be calculated from daily diaries during run-in and during the treatment periods. Weekly responders can be defined as patients in whom the average LPDS during a treatment week is lower than the run-in period by a margin of at least the

MCID. The exact responder threshold setting will depend on the expected magnitude of therapeutic effect and the need or desire to keep the placebo response lower or higher. The five other symptoms in the diary (epigastric pain, epigastric burning, nausea, belching, and heartburn) can be used to monitor changes, either improvement or worsening, in upper gastrointestinal symptoms that often accompany PDS.

Some supportive validity results were also obtained for several aspects of the EPS scores (epigastric pain and burning) in the diary, but based on the eligibility criteria these symptoms were less prevalent and of lesser intensity, leading to overall less strong numerical data. Hence, further separate focused studies will be required to validate an instrument for FD/EPS.

The draft Dutch LPDS questionnaire was meanwhile adapted into five languages using a standard method that involves forward and backward translations. However, cognitive interviews of PDS patients from each country and their evaluation are still necessary to confirm the linguistic validation.

We conclude that the LPDS is a sensitive and reliable instrument to assess severity of PDS symptoms, according to the Rome III definition, on a daily basis. The LPDS diary can be used to assess symptom fluctuations and treatment outcomes in research and clinical practice. The European Medicines has expressed its support to its use in clinical trials of patients with PDS (Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Other/2015/12/WC500198874.pdf; 29 January 2016).

3.4.1. Introduction

Functional dyspepsia (FD) defined as "the presence of symptoms thought to originate from the gastroduodenal region in the absence of any structural or metabolic disease that is likely to explain these symptoms" has been proposed to be subdivided in Epigastric Pain Syndrome (EPS) and Postprandial Distress Syndrome (PDS) (41, 42, 197). There is an urge to categorize patients that have a clear symptomatic pattern into the present subgroups for appropriate clinical management. However, in clinical practice, there is a large overlap between PDS and EPS (42, 53-55), therefore questioning the usefulness of the subdivision.

Furthermore, despite the fact of the high proportion of the PDS patients, there are limited treatments with established efficacy for PDS (119, 132, 222, 238, 239). This is probably due to the use of inappropriate endpoints or no validated instruments to assess of symptoms and their responsiveness (240).

Previously, we developed and validated with success a new Patient-Reported Outcome (PRO) questionnaire for the PDS subgroup, the Leuven Postprandial Distress Scale (LPDS), in line with regulatory guidelines (19). In this study FD patients were classified into the PDS subgroup, as in agreement with the Rome III criteria, if they reported bothersome postprandial fullness and/or early satiation at least several times per week.

A recent study has shown associations on meal-related symptoms in the "pure" PDS and a subgroup of PDS-EPS overlap patients. Furthermore, it was shown that by adapting the Rome III subdivision by taking into account the meal relationship of dyspepsia symptoms, reduces the overlap between PDS and EPS through an increase in an adapted PDS subgroup, hence indicating the relevance of the ingestion of a meal in triggering FD symptoms (169). Nevertheless, despite the symptomatic similarities in both populations, it has not yet been explored whether their psychometric properties are as well alike, therefore, making it difficult to use similar clinical management approaches. During the validation study of the LPDS PRO questionnaires, patients were also included if they reported non-predominant EPS symptoms, allowing some room for the inclusion of overlapping PDS-EPS patients. Hence, the aim of this study was to re-explore the validity and usefulness of the LPDS questionnaire in a "pure" PDS population and in an overlap PDS-EPS population. Moreover, this study could help us understand the impact of the PDS symptoms in the selected populations and its effect on the future strategy for patient selection.

3.4.2. Materials and Methods

Study design

This study was designed as an additional analysis of a double-blind randomized, multicenter, placebo-controlled study in PDS patients receiving either itopride 100 mg or placebo three times daily. However, the treatment medication was irrelevant to this study and the blind was not broken during this evaluation of the measurement properties of the LPDS. Use of a treatment trial allows the evaluation of responsiveness to change of the new symptom evaluation instrument (LPDS) by deliberately inducing change in some patients. After selection according to Rome III criteria for PDS, patients entered the eligibility period. During these 2 eligibility weeks, patients filled out the draft LPDS daily diary. If eligible based on the symptom pattern and frequency (see below), patients were then

randomized in a double-blind fashion into the 2 parallel treatment arms. During this period, all patients continued to fill out the LPDS daily diary and additionally the Overall Treatment Evaluation, Overall Symptom Severity, PAGI-SYM and SF-NDI questionnaires at the end of the run-in period and after 2, 4, 6 and 8 weeks of treatment. During this period, there were three outpatient clinic visits (visit 3, 4 and 6) and one telephone call (visit 5)

Patient selection

Consecutive outpatients diagnosed with PDS according to Rome III criteria at eleven gastroenterology practices in Belgium were eligible for the study. Both French and Dutch speaking patients between the ages of 18 and 70 years were included.

FD patients were classified into the PDS subgroup, as in agreement with the Rome III criteria, if they reported bothersome postprandial fullness and/or early satiation occurring after normal-sized meals at least several times per week.

Following these criteria, patients were included if they were confirmed to suffer from active PDS as per LPDS scoring system during the 2 weeks eligibility period (see below). This required the presence of at least moderate (score 2) postprandial fullness and/or early satiation symptoms on at least 4 days during the 2 weeks eligibility period.

Patients were excluded if they reported frequent and bothersome co-existent GERD symptoms, or if they had a history of reflux esophagitis. Furthermore, patients were excluded if they reported predominant symptoms of irritable bowel syndrome (IBS), chronic nausea (present every day), vomiting (more than once a month), excessive belching (present every day) and when patients presented symptoms of EPS several times a week according to Rome III questionnaire.

In addition, patients were excluded if they failed to fill out the questionnaires adequately. Female patients who were pregnant or lactating were also not eligible. Furthermore, patients were excluded if they had abnormal findings on upper gastrointestinal endoscopy, and if they had a history of upper digestive surgery, diabetes, coeliac disease, inflammatory bowel disease or any other disorder affecting upper GI motility such as dysphagia. Patients who were *H. Pylori* positive or patients who received treatment for HP eradication during the preceding 3 months were also excluded. Patients taking prohibited medication needed a wash-out period of 2 weeks prior to screening. Prohibited medications included those medications that could influence gastric physiology, motility and sensitivity or that could induce lesions in gut mucosa. Patients with an active psychiatric condition (major depression, anxiety disorder, alcohol or substance abuse) were excluded. However, patients who were taking a stable dose of a single antidepressant (except amitriptyline) FD during the last 3 months were eligible.

FD patients' subdivision

Patients were subdivided as "pure" PDS patients if they experienced postprandial fullness and/or early satiation occurring after normal-sized meals at least several times per week with no occurrence of bothersome epigastric pain. The overlap of PDS and EPS was defined as those patients suffering from postprandial fullness and/or early satiation occurring after normal-sized meals at least several times per week and epigastric pain at least than once a week. Furthermore, patients were asked if the epigastric pain was occurring most frequently after the ingestion of a meal.

Study drug

Itopride is a prokinetic benzamine derivative with dopamine-2 antagonistic and cholinesterase inhibitory properties, which generates a prokinetic effect on gastric motility (129-132, 222)

Questionnaires

Symptom severity was assessed with a daily diary and with questionnaires that were completed at fixed time points during the trial. The questionnaires used were the Leuven Postprandial Distress Scale (LPDS) diary, Overall Symptom Severity assessment, Overall Treatment Evaluation, the patient assessment of upper gastrointestinal symptom severity index (PAGI-SYM) and the short form-Nepean Dyspepsia Questionnaire (NDI)(213-216, 241).

Based on a literature survey, the Rome III definitions, focus group sessions and cognitive debriefing, a provisional diary instrument was constructed which consisted of 8 upper abdominal symptoms (early satiation, postprandial fullness, upper abdominal bloating, epigastric pain, epigastric burning, nausea, belching and heartburn) (19). The rating of the items is expressed as verbal descriptors (5 levels per item, ranging from absent to very severe) accompanied by "smiley faces" (☺ to ☹).

Weekly change of the symptoms was assessed by Overall Treatment Evaluation and Overall Symptom Severity scales. In the Overall Treatment Evaluation question, patients were asked to indicate on a 9-point scale if their dyspepsia symptoms had improved, remained unchanged or had worsened since the last evaluation before starting the treatment. In the Overall Symptom Severity question, patients indicate the overall severity of their dyspepsia symptoms in the last week on a 6-point likert scale (0, no symptoms present; 5, very severe symptoms) every 2 weeks (213, 217, 244, 245).

The PAGI-SYM has been developed and validated for the evaluation of therapeutic responsiveness in FD (213, 214). It consists of 20 items divided into 6 symptom subscales (heartburn/regurgitation, fullness/early satiety, nausea/vomiting, bloating, upper abdominal pain, and lower abdominal pain). Each item covers a range from 0 (none or absent) to 5 (very severe) and assesses symptom severity over the preceding 2 weeks; the subscales are obtained by averaging symptom group scores, also ranging from 0 to 5.

The NDI questionnaire is a disease-specific quality of life measure for dyspepsia. It addresses 10 items distributed over 5 domains (tension/sleep, interference, eating/drinking, knowledge/control, and work/study) to assess the impact of FD on the subject's quality of life. All items are measured by 5-point Likert scales from 0 (not at all or not applicable) to 4 (extremely)(211, 215, 216, 241).

Statistical analysis

The LPDS daily diary uses "smiley faces" (☺ to ☹) to score symptoms on an absent to very severe range. These were numerically transformed into a 0-4 score range, and averaged for each symptom as a weekly score (0-4). Evaluation of the LPDS measurement properties utilizes baseline measures (visit 2 of the trial) which were considered the most reliable visit to measure symptom pattern and severity, as this is at the end of an evaluation period off potentially interfering pre-existing therapies, and prior to the start of placebo-controlled treatment. The end of treatment measurements (visit 6 of the trial) were used for the purpose of responsiveness.

The LPDS is regarded as the test measure while a number of other instruments are used as external anchors (Overall Treatment Evaluation, Overall Symptom Severity, PAGI-SYM, NDI) in evaluating a

number of aspects of the validity and reliability of measurement of the LPDS, as previously described in the validation of LPDS in FD patients.
To examine the possibility that the instrument's psychometric characteristics vary between the PDS and EPS subgroups analyses have been conducted for the entire functional dyspepsia sample and within PDS and EPS subgroups.
The rationale to target the PDS-EPS overlap population in this study was mainly to explore the weight of the PDS symptoms in an overlapping cohort therefore, to determine the importance of sampling strategy in future clinical cohorts.

Construct validity

The construct validity of the LPDS was assessed by means of confirmatory factor analysis which specifies the domain structure of the LPDS in a latent variable model in which each domain of the instrument is represented by a latent variable and each of the symptom items are observed variables at study visit 2. If the a priori specified model reproduces the observed correlations between symptom items well this is taken as support for the construct validity of the instrument. CFA model fit targets have been adopted from Schermelleh-Engel et al (247) including the Chi-Square measure (ideally non-statistically significant), the ratio of Chi-Square to degrees-of-freedom (ideally <5), Goodness-of-fit (GFI, ideally >0.95), Comparative Fit Index (CFI, ideally >0.95) and Root Mean Square Error of Approximation (RMSEA, ideally <0.05). In addition, for the measurement model to support the hypothesized constructs all individual item loadings need to be statistically significant and consistent, although some variation in loading magnitude would not be surprising or harmful to the hypothesis.

Known groups (criterion) validity

Criterion validity was assessed by comparing groups which would theoretically be expected to differ in distribution of LPDS domain scores. For this purpose individuals scoring high (4-5) or low (1-3) on the Overall Symptom Severity scale were compared with respect to PDS and EPS scores using the Mann-Whitney test. A standardized measure of effect size, Cohen's d, is also reported to quantify the degree of differentiation between groups. Values of d>0.8 are generally considered 'large'.

Convergent validity

The convergent validity concept adds credibility to the LPDS by showing that it is related to other measures of dyspepsia symptom burden. The LPDS at visit 2 was correlated with the Overall Symptom Severity, with the NDI-SF and with the PAGISYM domains corresponding to early satiation and/or postprandial fullness, upper abdominal pain and lower abdominal pain. All NDI domains are potentially relevant but it was a priori hypothesized that the eat/drink domain of the NDI should yield the strongest correlation with the PDS domain of the LPDS. The LPDS would be supported as a new measure of dyspepsia burden if there are moderate, positive correlations with the other measures. If the correlations are too high (>0.9) the LPDS would not be adding new information over and above existing measures.

Internal consistency

The extent to which items within each LPDS symptom domain were related was assessed via the measurement model on the visit 2 measurements. A more direct assessment was also undertaken through measures of internal consistency. Descriptively the correlations between items were calculated and these are expected to be positive and high (>0.7). Cronbach's α was also calculated although this may not be as high as typically desired due to the small number of items per construct. Generally α values>0.6 or ideally>0.8 are sought.

3.4.3. Results

Study population

After eligibility evaluation, 99 PDS patients (79% females, 39.1±1.5 years old, 62.5±1.2 Kg and 1.68±0.4 m) were randomized. Of these, 92 completed the entire study and 7 patients dropped out of the study in the last weeks (week 6 or week 7), in general because of lack of efficacy of the treatment.

Rome III symptom frequency

All patients suffered from postprandial fullness (97%) and/or early satiation (73%) several times per week. Upper abdominal bloating and nausea was reported by 80% and 38% of the patients, respectively. Non-predominant and not bothersome EPS symptoms were allowed during the study. Epigastric pain several times a week, were reported by 18% of the patients. The Rome criteria classify patients with EPS if symptoms are occurring at least once a week. Epigastric pain occurring at least once a week was observed in 54% of the study population but this occurred mainly after meals.

When subdividing the FD patients in agreement with the Rome criteria, 45 patients were defined as "pure" PDS (70% females, 41.2±2.6 years old, 64.2±1.7 kg) and 54 patients were defined as patients overlapping PDS and EPS (81% females,37.4±1.8 years old, 61.1±1.6 kg). PDS symptoms were the dominant symptoms and in the overlap subgroup epigastric pain was mostly meal-related (90%) (Figure 1).

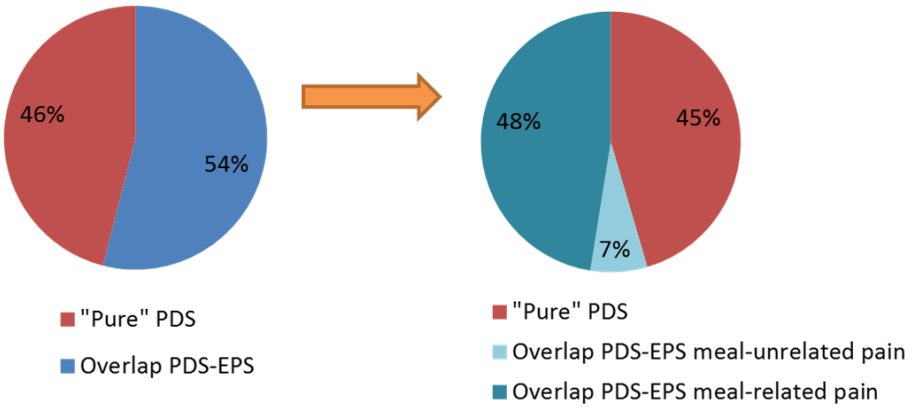


Figure 1. PDS and PDS-EPS overlap group. The graph on the left diagram shows the proportion of the 99 enrolled patients who fulfill Rome III criteria for pure PDS and those who fulfill both PDS and EPS criteria. The graph on the right illustrates that the co-existing EPS symptoms are related to meal ingestion in the vast majority of patients.

LPDS questionnaire

LPDS Diary scores 2-week eligibility period for postprandial fullness (2.2±0.1 vs. 2.5±0.1; p=0.06) and early satiation (1.8±0.1 vs. 2.2±0.2) were high for both the "pure" PDS as the overlap subgroup, respectively (p=0.06). Bloating (p=0.02) and epigastric pain (p<0.0001) were significantly higher in the overlap PDS-EPS subgroup. Epigastric burning (p=0.001) and heartburn (p=0.002) were less common but higher in the overlap subgroup (Figure 2). Similar low severity scores were observed for nausea (1±0.2vs.1.1±0.1;p=0.5) and belching (0.2±0.1 vs.0.7±0.1; p=0.6). Similar results were obtained for the baseline symptom severity levels as assessed by the PAGI-SYM questionnaire (data not shown).

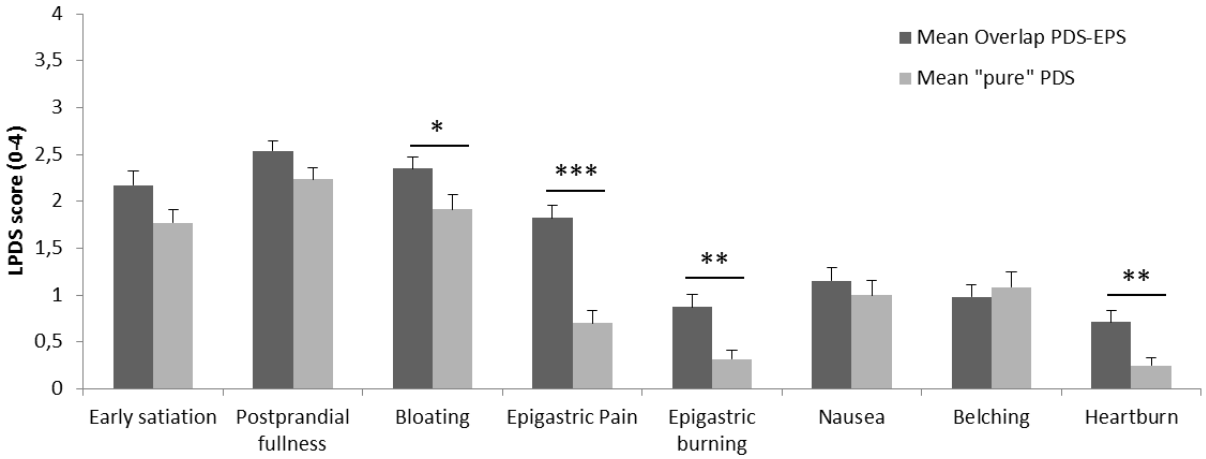


Figure 2. Mean severity score of LPDS symptom scores at the end of the 2-weeks eligibility period in "pure" PDS and Overlap PDS-EPS. *p<0.05; **p<0.01; *p<0.001

Construct validity 1: Latent variable structure

The two latent variable model hypothesized a priori in the LPDS validation study showed that the construct including early satiety, postprandial fullness and meal-related upper abdominal bloating form one hand and epigastric pain and epigastric burning on another hand provided sufficient fit to the data of visit 1 and 2, and hence, an adequate representation of the symptoms of functional dyspepsia. This construct showed as well a good fit after separation into "pure" PDS and overlap PDS-EPS (Table 1).

Table 1. Confirmatory Factor Analyses at Visits 1 and 2

Item	FD (n=98)	"Pure" PDS (n=45)	Overlap PDS-EPS (n=53)
Early satiety	0.76 (0.06)	0.57 (0.12)	0.84 (0.06)
Postprandial fullness	0.89 (0.05)	1.00 (0.10)	0.87 (0.05)
Bloating	0.69 (0.06)	0.59 (0.12)	0.73 (0.08)
Epigastric pain	0.94 (0.08)	1.09 (0.21)	0.88 (0.11)
Epigastric burning	0.68 (0.08)	0.62 (0.15)	0.65 (0.11)

Known groups (criterion) validity

Mean scores of PDS and EPS symptoms on the "pure" PDS and the overlap PDS-EPS mean scores were considered significantly elevated in patients whose Overall Symptom Severity score was in the high range (4-5) compared with those whose Overall Symptom Severity score was not high (1-3). Cohen's d effect sizes were in the large range (>0.8) for all groups (Table 2).

Table 2. Known groups (criterion) validity (visit 2) in 99 patients recruited for the validation study of the Leuven Postprandial Distress Scale (LPDS) and subdivided into "pure" PDS and overlap PDS-EPS. Scores for the PDS and the EPS symptoms in the LPDS diary are given as mean (SEM); p-values are based on the Kruskal-Wallis test. Cohen's d is a standardized measure of effect size. Values >0.8 are generally interpreted as 'large'.

Measure	Low OSS (1-3)	High OSS (4-5)	p-value	Cohen's d
FD (n=89)				
PDS	4.7 (1.8)	7.9 (2.3)	<0.0001	1.56
EPS	0.8 (1.2)	2.6 (1.9)	<0.0001	1.16
"Pure" PDS (n=41)				
PDS	4.9 (1.7)	7.4 (2.3)	0.001	1.25
EPS	0.4 (0.7)	1.5 (1.7)	0.03	0.92
Overlap PDS-EPS (n=48)				
PDS	4.4 (1.8)	8.2 (2.2)	<0.001	3.90
EPS	1.3 (1.5)	3.3 (1.7)	0.0003	1.25

Convergent validity

Cross-sectional correlations at visit 2 for the FD patients, "pure" PDS and the overlap PDS-EPS for the LPDS PDS and EPS scores on the one hand and Overall Symptom Severity, PAGI-SYM and NDI on the other demonstrated significant positive correlations. OSS correlated with LPDS domains more strongly with PDS than EPS in the general population as well as for "pure" PDS and the overlap subgroup. (Table3). For all groups, there was a strong correlation with the PDS score and for early satiation/postprandial fullness subscale of the PAGI-SYM as well as for the eat/drink subscale of the NDI. The reverse was observed for the PAGI-SYM upper abdominal pain subscale in the FD and "pure" PDS. This was moderated on the overlap subgroup.

Table3. Correlation analyses between LPDS and other questionnaires. Cross sectional (visit 2) correlations are reported as Pearson correlation coefficients. Analysis was replicated using non-parametric Spearman correlation with no substantive difference in magnitude.

	FD		Pure		Overlap	
	PDS	**EPS**	**PDS**	**EPS**	**PDS**	**EPS**
OSS	0.60 <0.0001	0.45 <0.0001	0.56 0.0001	0.31 0.046	0.61 <0.0001	0.47 0.0008
PAGI: PPF/ES	0.68 <0.0001	0.41 <0.0001	0.62 <0.0001	0.36 0.02	0.72 <0.0001	0.38 0.007
PAGI: Upper AP	0.41 <0.0001	0.68 <0.0001	0.19 0.2	0.51 0.0006	0.43 0.0002	0.65 <0.0001
PAGI: Lower AP	0.15 0.2	0.43 <0.0001	0.22 0.2	0.31 0.049	0.01 >0.9	0.43 0.002
NDI: Tension	0.32 0.002	0.42 <0.0001	0.13 0.4	0.15 0.3	0.38 0.006	0.53 <0.0001
NDI: Interference (ADL)	0.21 0.04	0.28 0.007	0.29 0.06	0.10 0.5	0.14 0.3	0.35 0.01
NDI: eat/drink	0.57 <0.0001	0.33 0.001	0.53 0.0004	0.17 0.3	0.55 <0.0001	0.32 0.03
NDI: knowledge/control	0.17 0.1	0.26 0.01	0.21 0.2	0.09 0.6	0.05 0.7	0.18 0.2
NDI: Work/study	0.25 0.02	0.26 0.01	0.34 0.03	0.28 0.08	0.17 0.2	0.19 0.2

Internal consistency

Cronbach's α was higher for the overlap PDS-EPS subgroup (0.85 at visit 2) compared to the pure PDS (0.74 at visit 2). α was moderate for EPS in the overlap group (0.72 at visit 2) and higher in the "pure" PDS group (0.81) (Table 4).

Table4. Internal consistency in PDS and EPS domain.

Item correlations	**FD (n=98)**	**"Pure" PDS (n=45)**	**Overlap PDS-EPS (n=53)**
ES/PPF	0.69 (<0.0001)	0.57 (<0.0001)	0.75 (<0.0001)
ES/Bloating	0.49 (<0.0001)	0.32 (0.03)	0.61 (<0.0001)
PPF/Bloating	0.61 (<0.0001)	0.59 (<0.0001)	0.63 (<0.0001)
PDS α	0.81	0.74	0.85
Epigastric pain/burning	0.66 (<0.0001)	0.68 (<0.0001)	0.57 (<0.0001)
EPS α	0.79	0.81	0.72

[A]***Correlations with full scale***

α is Chronbach's α and is reported in standardized form. Table cell entries are Pearson correlations and p-value

3.4.4. Discussion

One of the reasons for the limited drugs of proven efficacy for PDS is the lack of a validated PRO instrument available for the evaluation of symptom severity or treatment efficacy. We recently develop and validated a new outcome assessment tool for PDS, the Leuven Postprandial distress scale (LPDS) in line with regulatory guidelines. For the validation of this new instrument 60 PDS patients were randomized after a 2-week run-in period in an 8-week double-blind placebo-controlled trial of itopride 100 mg three times daily. However, for this study non-predominant EPS symptoms were allowed, therefore, leaving room for EPS overlap in the PDS population.

In this follow up study, 99 PDS FD patients were included showing overlapping EPS symptoms in 54% of the population. This overlap subgroup showed as the "pure" PDS population high rates of postprandial fullness and early satiation. Moreover, upper abdominal bloating, epigastric pain, epigastric burning and heartburn scores were significantly higher in the overlap subgroup compared to "pure" PDS.

Despite the slight differences in the "pure" and overlap PDS populations, the usefulness of the instrument persisted for both groups. When analyzing symptom prevalence profiles and factor analysis in both subgroups, the principal latent variable comprised the PDS symptoms of postprandial fullness, early satiation and upper abdominal bloating as previously shown. The EPS symptoms corresponded to the second latent variable.

In both subgroups, the mean LPDS diary ratings of the 3 PDS symptoms (postprandial fullness, early satiation, upper abdominal bloating) correlated well with known-severity groups, based on the patients' OSS ratings. Convergent validity of the instrument showed stronger correlation with the overlap PDS-EPS subgroup, but it was demonstrated in both groups. Finally, the internal consistency and reproducibility of the LPDS score in the "pure" PDS as well as in the overlap subgroup were confirmed.

From this data, we can conclude the validity and reliability of the LPDS instrument in the overlap PDS-EPS subgroup; therefore, indicating its usefulness in a larger FD patient set-up to assess symptom fluctuations and treatment outcomes in research and clinical practice.

Chapter 4

The assessment of intragastric pressure in functional dyspepsia patients and healthy subjects.

4.1.1. Introduction

Functional dyspepsia (FD) is one of the most common gastrointestinal disorders encountered in clinical practice (41, 167). Based on Rome III criteria, FD is defined as the presence of symptoms thought to originate in the gastroduodenal region (early satiation, postprandial fullness, epigastric pain or burning), in the absence of any organic, systemic or metabolic disease that is likely to explain the symptoms (41, 167). Several pathophysiological mechanisms have been proposed to underlie symptom generation in FD. The main hypotheses include visceral hypersensitivity due to central or peripheral sensitization, low-grade inflammatory states, altered secretion of gastrointestinal hormones, genetic predisposition and abnormal gastric emptying or accommodation.

The prevalence of impaired accommodation is about 40% in patients seen at tertiary referral centers (21, 22). Impaired gastric accommodation is associated with increased prevalence of symptoms of early satiety and weight loss (21, 22, 36, 163). A number of studies have indicated the importance of gastric accommodation in the control of food volume tolerance in FD patients but also in healthy volunteers (HVs) (20), supporting the hypothesis that impaired accommodation is an important mechanism underlying symptoms of early satiation and weight loss.

The drink test was developed as a minimally invasive, patient-friendly technique to discriminate FD patients from HVs (99, 251-253). The volume of nutrient drink consumed during a slow nutrient drink test has been proposed to serve as a surrogate parameter for gastric accommodation (162, 163). However, the specificity of impaired drinking capacity to reflect impaired accommodation has been questioned since decreased consumption can be attributed not only to impaired gastric accommodation, but also to delayed gastric emptying, antral distension, duodenal feedback mechanisms or taste aversion and only poor correlations have been reported with measures of gastric sensation, accommodation and emptying (252, 254).

The gold standard to determine gastric accommodation is the gastric barostat (59, 154, 255). This technique detects changes in muscle tone by measuring volume changes of an intragastric balloon that is kept at a constant pressure. However, a number of important drawbacks are associated with the technique: the inflated balloon distends the proximal stomach, may exaggerate gastric accommodation and hampers physiological responses to food intake (158, 256, 257). Moreover, muscle tone can only be measured in a limited stomach region and the invasive nature of this technique, which is perceived as uncomfortable and stressful, excludes its application in routine clinical practice. There is a clear need for an alternative technique to assess gastric accommodation during food intake.

We recently reported a technique for assessment of gastric accommodation during food intake in HVs by measuring the IGP (160, 258). Moreover, this IGP measurement has also been applied to quantify the influence of different pharmacological agents in HVs (166, 259). With this method, relaxation of the stomach is accompanied by an IGP decrease and when gastric accommodation is impaired through inhibition of nitric oxide synthase, IGP during food intake is elevated and nutrient tolerance is decreased (14). Using this technique, we observed that the IGP drop and its subsequent recovery during nutrient drink ingestion in HVs is significantly correlated to satiation and the volume required to induce maximal satiation (13;14). In the present study, our aim was to investigate differences between FD patients and HVs by measuring the IGP during intragastric nutrient drink infusion.

4.1.2. Materials and Methods

Patient and volunteer selection

All study procedures were approved by the Ethics Committee of the Leuven University Hospital, Belgium.

Consecutive patients were selected from the outpatient clinic pool of patients presenting with epigastric symptoms. Inclusion criteria were the presence of unexplained regular meal-induced dyspeptic symptoms (bothersome post-prandial fullness after an ordinary sized meal that occurs at least several times per week or early satiation that prevents the finishing of a regular sized meal at least several times per week). One or both of these must have been present for at least the last three months with an onset of symptoms at least six months prior to diagnosis (41, 167). Organic, systemic or metabolic disease were excluded as possible explanation for the symptoms by means of upper gastrointestinal endoscopy, routine biochemistry and upper abdominal ultrasound, performed as routine clinical examinations. Other inclusion criteria were the willingness to sign the informed consent, age between 18 and 75 years and a body mass index (BMI) < 32 kg/m^2. Exclusion criteria were predominant heartburn, the presence of esophagitis, gastric atrophy or erosive gastroduodenal lesions on endoscopy, heartburn as a predominant symptom, a history of peptic ulcer, major abdominal surgery, underlying psychiatric illness and the use of non-steroidal anti-inflammatory drugs, steroids or drugs affecting gastric acid secretion. All drugs potentially affecting gastrointestinal motility or gastric acid secretion were discontinued at least 1 week prior to the study.

Healthy volunteers were recruited through local advertisement. None of the HVs had symptoms or a history of gastrointestinal disease, other significant diseases, psychological disorders or drug allergies; none were taking any medication or had any drug history.

All subjects participated after an overnight fast, and they were asked to refrain from alcohol, tea and coffee at least 12 hours before participation, and to refrain from smoking cigarettes at least 1 hour before the start of the experiment.

IGP measurement during nutrient drink infusion

Preparation of the subjects

The experimental procedure has been described in detail previously (160). In short, after an overnight fast, a high-resolution solid-state manometer system (36 channels, 1 cm in between each channel, Manoscan 360, Sierra Scientific Instruments, Los Angeles, USA, Manoview analysis software v2.0.1) was positioned through the nose so that at least 1 sensor was positioned in the lower esophageal sphincter (LES; detected as a clearly elevated pressure zone compared to oral and aboral areas), while IGP was measured as the average pressure of the first 5 pressure channels that were clearly positioned below the LES or the pressure area influenced by the LES (approximately 3-8 cm under the LES). A second catheter (Flocare, Nutricia, Bornem, Belgium) was positioned in the stomach through the mouth and was used to infuse nutrient drink directly into the stomach. The tip of the infusion catheter was positioned approximately 5 cm under the LES and its position was verified by fluoroscopy. The catheters were fixed to the subject's chin.

General protocol

After positioning of the catheters the subjects were seated in a comfortable position with the knees bent (80 degrees) and the trunk upright in a specially designed bed. Following a stabilization period of

at least 30 minutes, nutrient drink (Nutridrink, Nutricia, Zoetermeer, The Netherlands; 630 KJ, 6 g proteins, 18.4 g carbohydrates, and 5.8 g lipids per 100 ml) was infused directly in the stomach at a constant speed of 60 ml min^{-1} determined by an automated system using a peristaltic pump (the subjects were unaware of this speed). During nutrient drink infusion the subjects were asked to score their satiation at 1-minute intervals, using a graphic rating scale that combines verbal descriptors on a scale graded of 0–5 (1, threshold; 5, maximum satiation). At 5-minute intervals the subjects were asked to fill out a visual analogue scale (100 mm) for 8 epigastric sensations (hunger, expected amount to eat, bloating, fullness, nausea, belching, abdominal cramps, epigastric pain). Intragastric infusion was stopped as soon as the subjects scored maximally on bloating, fullness, nausea, belching abdominal cramps or epigastric pain or when a score of 5 was reached on their satiation scores.

Data analysis

The original data were imported from the recording software to Excel. We were primarily interested in slow IGP changes that could reflect changes in gastric muscle tone. Therefore, and in order to avoid influence from movement artefacts, a moving median was calculated per channel from the original data (median value over 1 minute of original data). Per channel, a baseline value was calculated from the moving median data as the average pressure in the last 5 minutes of the stabilization period. IGP data were presented per minute as the difference of the moving median value in that minute and the baseline value and as the average value of the 5 measurement channels that were clearly positioned below the LES as described above. The nadir IGP was defined as the lowest IGP measured during the nutrient drink infusion, and the delta-IGP as the change in IGP between the baseline and the nadir IGP. All continuous data were presented as mean ± standard error of the mean (SEM) and compared with Student's t-test, or 2-tailed Pearson correlations where appropriate.

4.1.3. Results

Patient and healthy volunteer characteristics

Sixty nine FD patients (76% were females, BMI of 22.2±1.4 kg m^{-2}) participated in the study. We also recruited 33 healthy subjects (67% were females, BMI of 22.5±0.5 kg m^{-2}). FD patients were significantly older than healthy controls (22.5±0.5 years old vs. 38.8±2.5 years old; p<0.0001).

IGP during intragastric nutrient drink infusion

During nutrient drink infusion the IGP decreased initially but gradually increased again upon continuous infusion (Figure 1.A.). Nadir IGP was reached later in HVs compared to FD patients (6.6±0.6 vs. 4.7±0.4 minutes after the start of the nutrient drink infusion, respectively, p=0.003). At the nadir, IGP was significantly lower in HVs compared to FD patients (-7.2±0.5 vs. -4.5±0.3 in FD patients, reflecting delta-IGP values of 7.0±1.3 and 4.5±0.3 mmHg from baseline in HVs and FD respectively, P<0.0001). When maximum satiation was reached the IGP was -5.7±0.5 in HVs and -3.1±0.4 mmHg and FD patients (p<0.0001).The mean area above the IGP curve (AAC) was significantly greater in the HVs group compared to FD (-5.57±0.5 vs. -2.9±0.3 mmHg*min, respectively, p<0.0001) (Figure 1.B.).

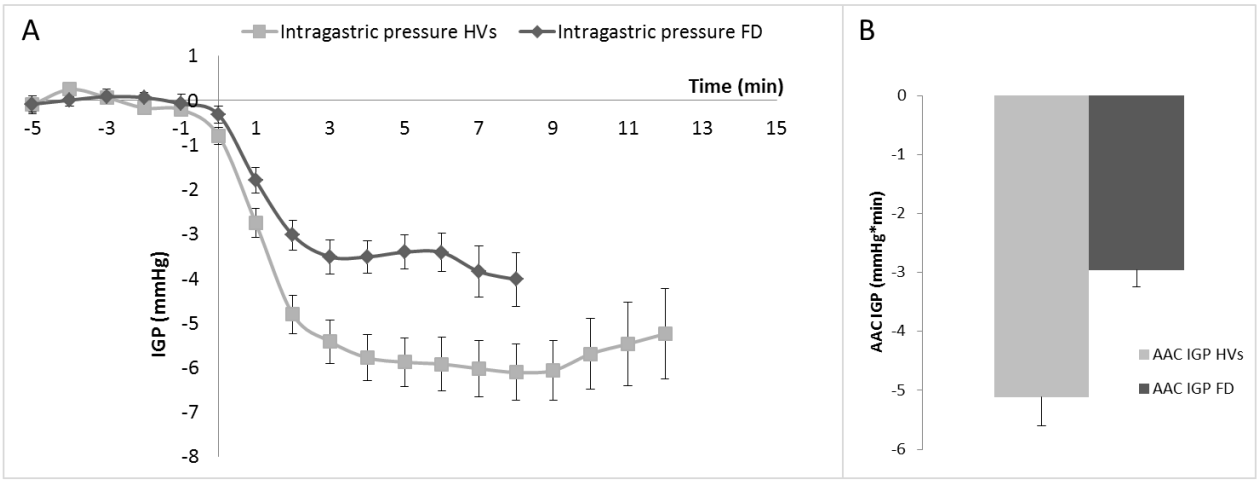


Figure 1. A. Time course of IGP before and during the intragastric infusion of a nutrient drink (1.5 Kcal per ml) in healthy subjects (HVs) and functional dyspepsia patients (FD). Data is shown after subtraction of the baseline value (calculated in the 5 minutes before nutrient drink infusion) until 50% of the subjects in each group reached maximum satiation. AAC values between FD patients and healthy subjects (HVs). The AAC was significantly larger in the HVs compared to the FD patients (p<0.0001).

Satiation during intragastric nutrient drink infusion

Satiation increased linearly from the start of the nutrient drink infusion with 0.45±0.04 and 0.84±0.2 units per minute, or 0.5±0.05 and 0.9±0.08 units per 100 kcal, respectively in HVs and FD patients until maximal satiation (p=0.006). Nutrient tolerance was significantly lower in FD compared to HVs. The volume consumed at maximal satiation was 723.6±51.2 ml or 1085±76.8 Kcal in HVs and 497.4±35.2 ml or 746.2±52.8 Kcal in FD patients (p<0.0001) (Figure 2).

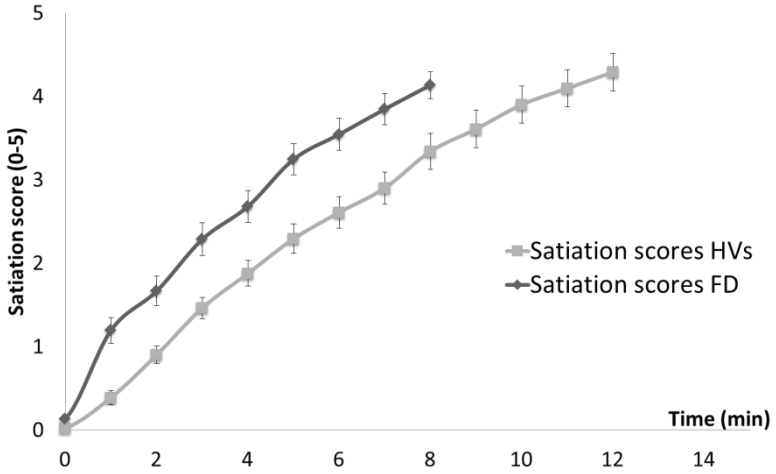


Figure 2. Time course of satiation score (0= no feeling of satiation, 5= maximal satiation) during the intragastric infusion of nutrient drink (1.5 Kcal per ml). Nutrient tolerance was significantly decreased in FD compared to HVs (p<0.0001). Data is shown as mean±SEM until 50% of the subjects in each group reached maximum satiation.

Epigastric symptoms before and during intragastric nutrient drink infusion

Before the meal, hunger scores were significantly lower in FD patients compared to healthy subjects (VAS score: 58 vs. 32, p<0.0001). Moreover, patients reported higher levels of fullness (VAS score: 14 vs. 5, p=0.05), bloating (VAS score: 10 vs. 2, p=0.03), nausea (VAS score: 11 vs. 1, p=0.005) and epigastric pain (VAS score: 9 vs. 0, p=0.003). After the intragastric infusion of the nutrient drink until maximal satiation, all symptoms were significantly increased in patients compared to healthy subjects (Figure 3).

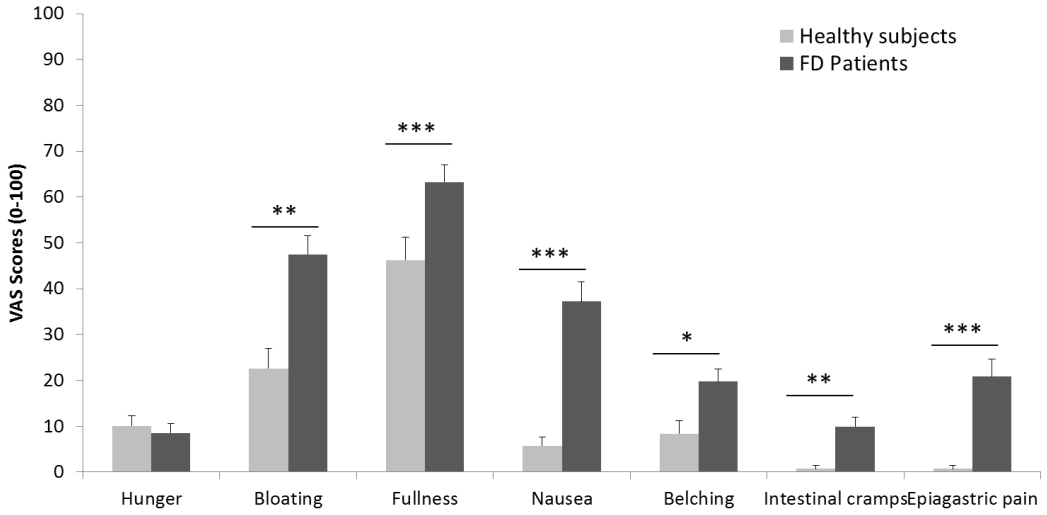


Figure 3: VAS scores of epigastric symptoms after intragastric infusion of nutrient drink until maximal satiation in healthy subjects and FD patients. All epigastric symptoms scores were increased in FD patients compared to healthy subjects. *p<0.05, **p<0.01, ***p<0.001

Correlation with symptom pattern

In the entire subject cohort, maximum tolerated nutrient volume showed a significant inverse correlation with nadir IGP (r=-0.35, p=0.0003).

When HVs and FD patients were assessed separately, the maximum ingested volume did not correlate well with the nadir IGP (r=-0.24, p=0.18 and r=-0.24, p=0.04, respectively).

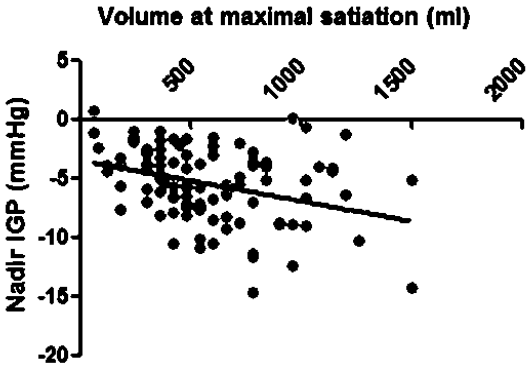


Figure 4: Spearman correlation of the volume at maximal satiation and nadir IGP r=-0.35, p=0.0003 or the area above the IGP curve over time (r=0.34, p=0.002) in all subjects.

In the entire population, modest correlations were observed between epigastric symptom scores such as epigastric pain, nausea and abdominal cramps after the meal and nadir IGP (Epigastric pain: r=0.43,

p<0.0001; Nausea: r=0.38, p=0.0004 and Cramps: r=0.32, p<0.004) (Figure 5). Moderate negative correlations were found between epigastric pain (r=-0.24. p=0.03) or nausea (r=-0.24. p=0.03) and maximal ingested volume. No correlations were found with epigastric symptoms and nadir or maximal ingested volume in the FD patients alone.

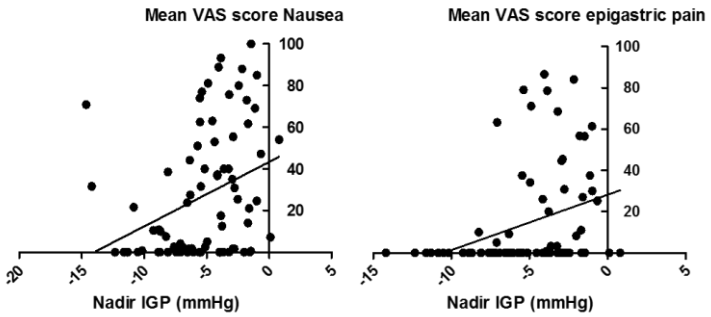


Figure 5: Spearman correlation on nadir IGP with mean VAS scores of nausea (r=0.38, p=0.0004) and epigastric pain (r=0.43, p<0.0001) during the intragastric infusion of a nutrient drink (300 Kcal).

Predictive value of IGP parameters and maximum tolerated volume for FD

The 10th percentile for maximum tolerated volume of nutrient drink was 384 ml in HVs. Using this cut-off, 31/69 (45%) of FD patients were outside of the HVs range. With regard to delta-IGP, the 10th percentile corresponded to a drop of 3.22 mmHg in the HVs group. When using this cut-off, this included 23/69 (33%) of the FD group. A quart of the patients (25%), showed a decreased nutrient tolerance (<384 ml) without an increased nadir IGP, and 13% of the patients showed an increased nadir IGP without a decrease nutrient tolerance. Using combined criteria of maximum tolerated volume ≤384 ml and a delta-IGP ≤3.22 mmHg, 16% of the all subjects (n=102) showed an abnormal IGP drop with decreased nutrient tolerance, of which 2% were healthy subjects and 16% were FD patients.

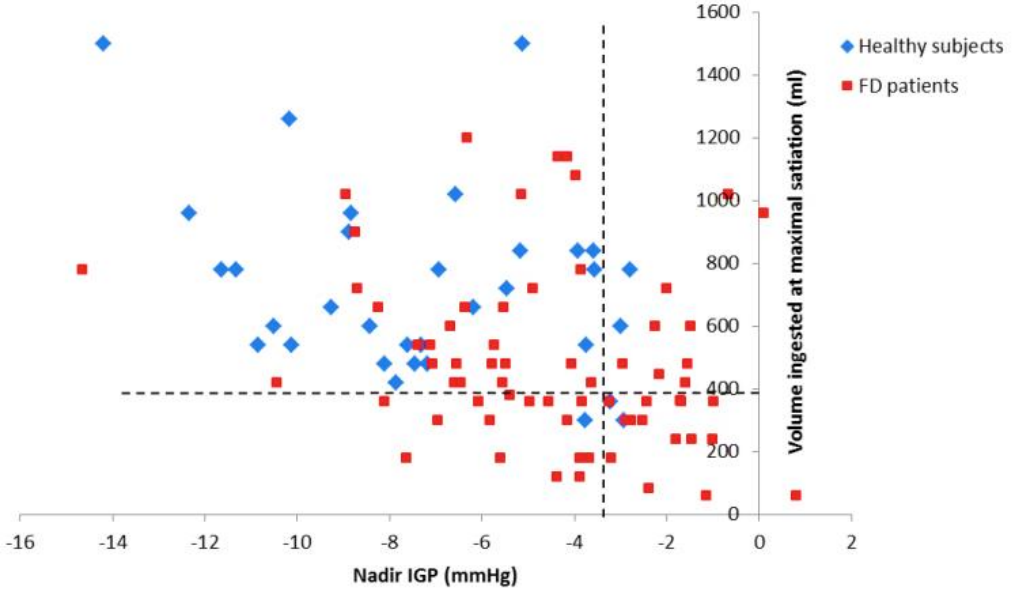


Figure 6. Description of the distribution of maximal tolerated nutrient drink volume and correspondent IGP drop during the intragastric infusion in all the subjects (69 FD patients and 33 healthy subjects).

4.1.4. Discussion

In this study we compared IGP and satiation during intragastric nutrient drink infusion in FD patients and HVs. IGP decreased initially during nutrient drink infusion and gradually increased upon continuous infusion in both HVs and FD patients. However, FD patients showed lower nutrient tolerance and a significantly lower drop in intragastric pressure during the intragastric infusion of the liquid meal compared to HVs. Patients experienced less hunger and reported more symptoms of fullness, nausea, pain and bloating before the meal. The ingestion of the meal aggravated or originated most epigastric symptoms in FD patients compared to HVs.

When including all subjects, it was observed that the lower the nadir IGP the more nutrient drink volume seemed to be ingested at maximal satiation. A moderate but significant correlation was seen between nadir IGP and severity of early satiation, suggesting that subjects with more severe meal-induced satiation score are more likely to have a smaller change in IGP with nutrient drink infusion, and by extension, poor gastric accommodation.

The barostat is considered the gold standard technique for assessing gastric accommodation, but it is also regarded as unphysiological and bothersome (158, 256, 257). Drink tests have also been used as a non-invasive measure of accommodation in patients with dyspeptic complaints. Studies confirm that, as a group, FD patients have decreased nutrient tolerance (162, 163, 171, 251, 253, 260). While impaired accommodation has been implicated as an underlying mechanism for this finding, gastric sensitivity and duodenal feedback, but also taste effects and psychological factors may play a role (162, 163, 252, 254).

In the present study we combined intragastric nutrient drink infusion with IGP measurement. This technique is based on the principle that the gastric muscle tone is known to decrease during food intake (increase of intragastric content) to provide a reservoir for the ingested food without IGP rise (21-24). When gastric accommodation is impaired, the muscle tone does not decrease normally; therefore, IGP is higher for the same intragastric volume leading to increased visceral sensation and feelings of satiation (6). We previously showed that IGP during nutrient drink infusion represents a measure of gastric accommodation and that the IGP is higher during nutrient drink ingestion when gastric accommodation is impaired (160, 258). In contrast to the typical nutrient drink test where the patient drinks the test liquid from one or two beakers, we chose to infuse the nutrient directly in the stomach. Hence, subjects were unaware of the infusion speed, could not taste the nutrient drink, did not have to swallow and could not estimate ingested volumes. We therefore bypassed any influence from taste (aversion) or subjective determinants of satiation and believe this method represents the most objective measurement of satiation. The findings of an association between earlier meal-induced satiation, decreased nutrient volume tolerance and smaller IGP drop in the present study are in agreement with previous study from our group, where we found a close association between impaired gastric accommodation and early satiation as well as weight loss in FD patients (4), although others failed to confirm this association (7,12).

A high variability was observed in this sample, in keeping with the presumed pathophysiological heterogeneity in FD (16, 57, 207). Forty-five per cent of FD patients ingested a maximum nutrient volume below the 10^{th} percentile in HVs. The difference in nutrient volume tolerance between HVs and FD patients is comparable with findings of previous studies using nutrient drink tests (25,7). Nadir IGP was explored as an additional criterion for diminished gastric accommodation. When nadir IGP was below 3.22 mmHg 38% FD patients were identified as abnormal. By combining both cut-offs in the

entire cohort, only 16% of the patients were characterized as abnormal, while this was found in only 2% of the HVs. Higher symptoms of pain, nausea and intestinal crams during the intragastric infusion of the nutrient drink were associated with a higher nadir IGP, indicating that a reduced drop in IGP is associated with more severe postprandial epigastric symptoms. Overall, these data indicate that intragastric pressure during a nutrient drink test has the potential to evaluate clinically relevant (patho-)physiological factors in FD.

This method uses the same manometry device as for standard assessments of esophageal motility; therefore, it has the potential to gain similar acceptance and feasibility level as an esophageal manometry. The technique is easy to perform, safe and well tolerated in healthy controls and patients and recently is has been introduced in FD pediatrics patients (261). The IGP measurement by HRM has significant advantages over other techniques providing simultaneously a great amount of information about gastric tone in fasted and fed state, nutrient volume tolerance, and permits the simultaneous recording of pressures in different portions of the stomach but also in the distal esophagus and proximal duodenum.

In summary, the intragastric nutrient drink infusion test with registration of IGP provides clinically relevant (patho-)physiological information by identifying subgroups with impaired accommodation or with decreased nutrient tolerance in FD. Although additional larger scale validation is needed, the test has the potential to provide a clinically applicable assessment of gastric accommodation and nutrient volume tolerance in patients with FD and other gastric motility disorders, including gastroparesis. Further studies are required to establish whether this test helps to discriminate FD from other upper GI conditions.

4.2. Impaired gastric distribution of a meal is associated with impaired meal-induced intragastric pressure (IGP) drop and early satiation in functional dyspepsia (FD).

4.2.1. Introduction

During ingestion of a meal, the gastric accommodation (GA) reflex generates a relaxation of the proximal stomach, which provides a reservoir for the storage of ingested food without a rise in intragastric pressure (IGP). In the past years, the reservoir function of the proximal stomach has mainly been studied by means of the gastric barostat, which can be considered the gold standard. The barostat technique detects changes in gastric tone by measuring the volume changes of a large intra-gastric balloon while its pressure is kept constant(255, 262). However, this method is very invasive and it may interfere with normal physiologic gastric responses due to the direct contact of the distended gastric balloon on the gastric wall (79, 155, 157, 158). Subsequently, a slow drinking test has been proposed as an alternative to estimate gastric accommodation by measuring nutrient tolerance, but this test may be influenced by taste and psychological factors (22, 36, 251, 260, 263-265). Imaging studies, using scintigraphy or ultrasound, have shown a redistribution of the meal to the distal stomach in FD patients, suggested to represent a consequence of impaired accommodation in the proximal stomach (195).

Recently, we have used intra-gastric high resolution manometry (HRM) to assess gastric pressure responses to meal intake. We observed that ingestion of intra-gastric infusion of a liquid meal is associated with an initial drop in intra-gastric pressure (IGP), followed by a progressive rise. Based on its inhibition by a nitric oxide synthase inhibitor, we proposed that the drop in IGP reflects gastric accommodation, and hence probably coincides and evolves with accumulation of the meal in the proximal stomach(160, 258). However, the relation between changes in intragastric pressure, the intra-gastric distribution of a meal and meal-induced satiation has not yet been studied in detail.

The aim of this study is to correlate the changes in intragastric pressure measurement with the gastric distribution of a liquid meal. Moreover, we want to analyze the correlation between changes in intragastric pressure during the gastric accommodation reflex on one hand and gastric distribution of a meal and maximal nutrient tolerance.

4.2.2. Materials and Methods

Subjects and study design

Healthy volunteers (HVs), recruited by public advertisement, were invited to participate in the study. HVs had to be devoid of GI symptoms and of the use of medications known to influence the GI motility. The study was approved by the Ethical Committee of the University Hospitals, Leuven, Belgium.

Consecutive outpatients diagnosed with FD according to Rome III criteria were eligible for the study. All patients completed the Rome III questionnaire and a previously validated gastro-esophageal reflux disease (GERD) questionnaire (194). Patients were excluded if they reported frequent and bothersome co-existent GERD symptoms, or if they had a history of reflux esophagitis. Furthermore, patients were excluded if they reported predominant symptoms of irritable bowel syndrome (IBS). Female patients who were pregnant or lactating were also excluded. Furthermore, patients were excluded if they had abnormal findings on upper GI endoscopy, and if they had a history of upper digestive surgery, diabetes, coeliac disease, inflammatory bowel disease or any other disorder affecting upper GI motility

such as dysphagia. The use of medication known to influence GI motility such as PPIs, needed to be washed-out period of 2 weeks prior the study.

The study consisted of a single visit of approximately 3 hours, during which the intragastric pressure (IGP) was assessed by means of a high-resolution manometry (HRM) and gastric volume changes were assessed by scintigraphy imaging. All subjects were requested to fast for at least 6 h before the study. Furthermore they were instructed to refrain from alcohol, tea and coffee at least 12 h before participation, and to refrain from smoking cigarettes at least 1 h before the start of the experiment.

A high resolution manometry probe (HRM, 36 pressure measurement points, ManoScan 360, Sierra Scientific instruments, Los Angeles (USA)) was passed through the nose into the distal stomach of the subjects. To allow direct infusion of a nutrient meal into the stomach, a nasogastric feeding tube (EnteralTM, Maxter-catheters, Marseille, France) was also positioned through the nose into the proximal stomach (maximum 5 cm below the LES). The position of the catheters was verified by fluoroscopy. The catheters were fixed to the subjects' nose and the subjects were placed on a sitting position with the trunk upright under a gamma camera (Siemens Gammasonics Inc., ECAM model, Germany, Erlangen) (Figure 1).

The IGP baseline was measured for at least 15 min. Hereafter, a bolus of radiolabeled (1 ml, 1mCurie of ^{99m}Tc-DTPA) saline was first intragastrically infused through the feeding tube and this was immediately followed by the intragastric infusion of nutrient drink (Fortimel Energy®, Nutricia, 150 kcal per 100 ml with 5.9 g proteins, 18.4 g carbohydrates and 5.8 g lipids, Netherlands) at a constant speed of 60 ml per minute. During nutrient drink infusion, subjects scored their satiation at 1-minute intervals using a graphic rating scale that combines verbal descriptors on a scale graded from 0–5 (5, maximum satiation). Intragastric infusion was stopped as soon as the subjects reached the maximum score of 5 on their satiation scale or when they scored maximally on one of the epigastric symptoms. Forty five minutes hereafter, the catheters were disconnected and removed (Figure 1).

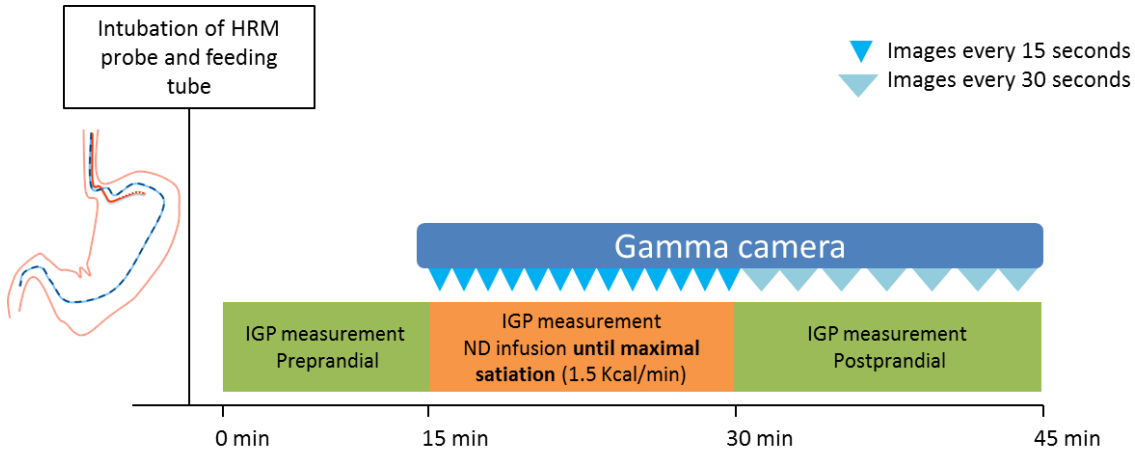


Figure 1. Study design. Time course IGP and scintigraphy measurement. After 15 min baseline IGP measurement, anterior and posterior images were taken during and after the intragastric infusion of a radioactive liquid nutrient drink.

During intragastric nutrient drink infusion until the end of the study, scintigraphy planar anterior and posterior images were obtained every 15 seconds for the first half hour and then every 30 seconds until the end of the study (Figure 1).

During the entire study, volunteers were asked to fill out visual analogue scales (VAS) for hunger, satiation and 6 epigastric symptoms (fullness, nausea, belching of air, cramps in the abdomen, bloating and pain) at 5-minute intervals.

Data Analysis

Intragastric pressure measurement study

The IGP original data was imported from the recorder software ManoAcquisition® to Excel. The data was calculated as previously described by Janssen et al (160, 258).

The IGP in the proximal stomach was measured as the average pressure of the first five pressure channels that were clearly positioned below the lower esophageal sphincter (LES) or the pressure area influenced by the LES. Similarly, the IGP in the distal stomach was measured as the as the average pressure five pressure channels that were clearly positioned in the distal part of the stomach, characterized by the presence antral contractions during the fasted state.

To avoid influence from movements such as swallowing, moving, etc., a moving median was calculated from the original data (median value over 1 minute of the original data). Per channel, a baseline value was calculated from the moving median data corresponding to the minimum pressure in the last 5 minutes of the stabilization period before nutrient drink infusion.

The nadir IGP was defined as the minimal IGP or the lowest relaxation point during nutrient infusion. Also, the area above the IGP curve (AAC) was calculated. Mean AUC satiation scores curves and the mean volume and time to reach maximal satiation were compared with the paired t-test. The data was used up to the time point where >80% of the evaluated subjects were still receiving nutrient infusion. The slopes of the IGP curve to nadir and from nadir back to baseline were calculated by linear interpolation and compared between groups with the non-parametric Mann–Whitney U test.

The visual analogue scales for epigastric symptoms (fullness, nausea, belching of air, cramps in the abdomen, bloating and pain) at 5-minute intervals for the FD patients and healthy control groups were compared with non-parametric Mann–Whitney U test. In all analyses $p < 0.05$ was considered significant.

Scintigraphy

Analysis of the images was done using the image processing tool PMOD 3.0. Anterior and posterior images were corrected for the difference of tissue attenuation and fused to compute their geometric mean. Time-activity curves corrected for isotope' physical decay were calculated by first averaging the geometric mean of the images and outlining of the volumes or regions-of-Interest (ROIs).

Four geometric ROIs were defined. A square defined the stomach and, below it, a rectangle, the duodenum. The diagonal on the square subdivided the stomach into the proximal and distal portion (Figure 2).

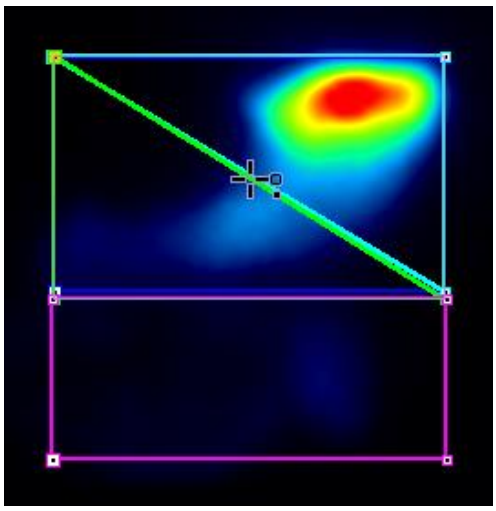


Figure 2. The geometric regions of interest were the stomach, subdivided into the proximal and distal part, and the duodenum.

The distribution of the radiolabeled nutrient volume within each ROI over time was calculated by multiplying the counts of the time-activity curve of each ROI by the ingested volume (known from the 60 ml/min infusion rate) per time point and, then, assuming equal distribution of label over the regions, by dividing it by the total amount of counts (sum of all ROIs) at every time point until maximal satiation. After reaching the end of the infusion, at the time of maximal satiation, the volume was considered constant for calculations of later time points. Gastric emptying was plotted as the residual fraction of radioactive nutrient drink in the stomach (proximal + distal) compared to the total (proximal + distal + duodenal).

Relationship between IGP and scintigraphy

Time series of continuous variables (e.g. intragastric distribution ratio, intragastric pressure (IGP) measurements) were controlled for normality and compared using Student's t-test and non-parametric Mann–Whitney U test. Correlations between variables were evaluated using linear regression analysis and Spearman's correlation. Proportions of the two groups were compared by using Chi-squared analysis. In all analyses $p < 0.05$ was considered significant.

4.2.3. Results

Study subjects

Healthy subjects

Fourteen healthy subjects (57% females, 23.6±0.7 years old, BMI: 23.2±0.9 $Kg.m^2$) were recruited for this study. All subjects gave informed consent before entering the study. One healthy subject was omitted from the analysis due to technical issues with the catheter observed in the IGP measurement. None of the subjects had symptoms or a history of gastrointestinal disease or drug allergies, nor were they taking any medication that could interfere with gut motility.

FD patients

Fifteen FD patients (86% females, 34.8±3.1 years old, BMI: 21.1±0.8 Kg.m^2) were recruited for this study. All the subjects gave informed consent before entering the study. All patients had a history of FD symptoms of at least 6 months. The most common symptoms were postprandial fullness (87%) occurring several times per week, upper abdominal bloating (87%) occurring several times per week and epigastric pain (73%) occurring at least once per week. In 90% of these patients, the epigastric pain was triggered or worsened by ingestion of the meal. Belching and nausea occurred several times per week in 53% and 60% of the patients respectively, and in 88% of these patients nausea was related to ingestion of a meal. Epigastric burning and reflux symptoms were the least commonly reported (7%) (Figure 3).

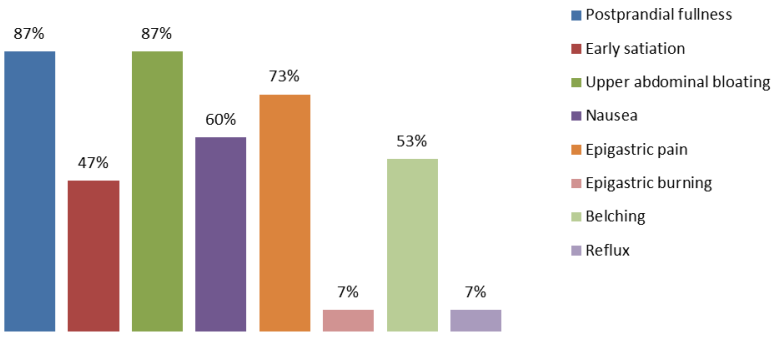


Figure 3. Symptom frequency in the last 6 months of FD patients

Intragastric pressure measurement

Satiation curve

Functional dyspepsia patients showed earlier satiation and tolerated less calories compared to healthy subjects. The average time to reach maximal satiation in HVs was 11.5±0.6 minutes and they received 688.8±37.4 mL or 1033.3±56.1 Kcal intragastrically. In FD the average time to reach maximal satiation was significantly shorter compared to HVs (7.6±1.1 minutes, p=0.004) and they received significantly less nutrient volume (454.1±63.6 mL or 681.6±95.4 Kcal, p=0.004). In agreement with these averages, the slope of the satiation curve was significantly steeper in FD patients compared the volunteers (slope HVs: 0.39±0.0 min^{-1} and slope FD: 0.66±0.1 min^{-1}, p=0.02). Furthermore, the area under the satiation curve was larger in the FD patients compared to HVs (AUC HVs: 124.9±8.4 min vs. AUC FD: 67.6±12.0 min, p=0.0007) (Figure 4).

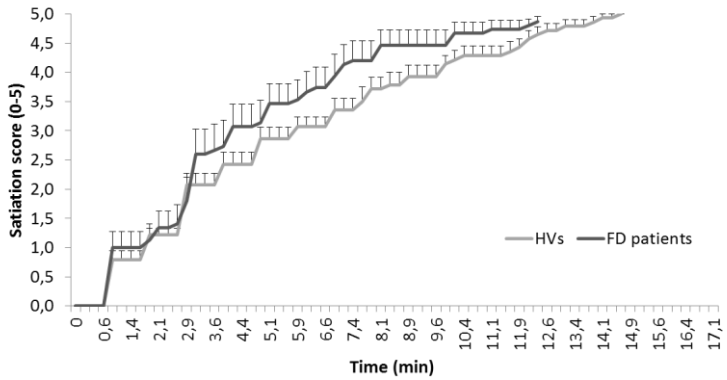


Figure 4. Time course satiation score of FD patients and healthy subjects.

IGP in the proximal stomach

During intragastric infusion of nutrient drink until maximal satiation, the IGP in the proximal stomach dropped from baseline in both groups. The drop in IGP in the proximal stomach in FD patients was significantly smaller compared to HVs (AUC until maximal satiation, HVs: -244±30.1 vs. FD: -111±37.4 mmHg*min, p=0.003). FD patients reached maximum satiation significantly earlier than healthy subjects (see also above) (Figure 5). To compensate for subjects reaching maximal satiation at different time points, the analysis was conducted at the time point where 80% of the patients were still receiving intragastric nutrient infusion (3.63 min, AAC FD=-36.6±11.1 vs. HV=-57.6±5.9 mmHg*min, p=0.07) and 100% (2.63 min, AAC FD=-25.5±7.9 vs. HV=-35.1±4.2 mmHg*min, p=0.15) of the patients were still receiving intragastric nutrient infusion.

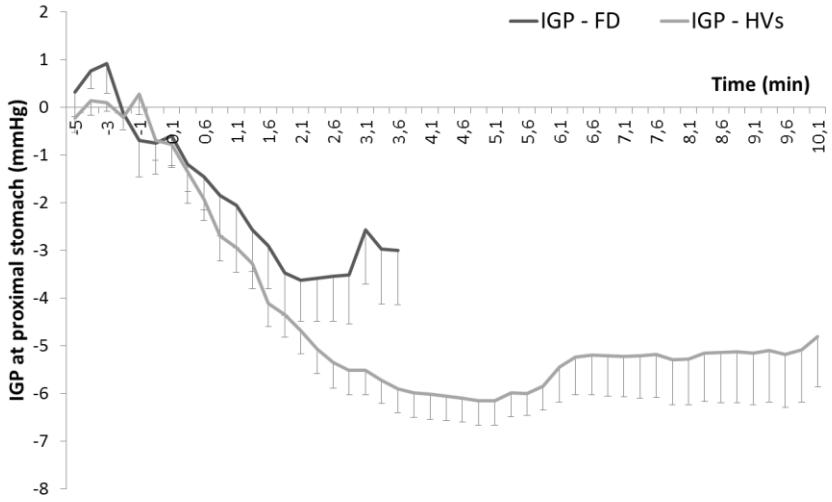


Figure 5. Time course of IGP in the proximal stomach during nutrient infusion until maximal satiation in FD patients and healthy subjects.

For all subjects, the area above the IGP curve (AAC) until maximal satiation in the proximal stomach was significantly correlated to the ingested volume at maximal satiation (n=28; r=-0.77, p<0.0001). When analyzed separately, a significant correlations were also found in FD (n=15; r=-0.58, p=0.02) and HVs (n=13; r=-0.64, p=0.02) (figure 6).

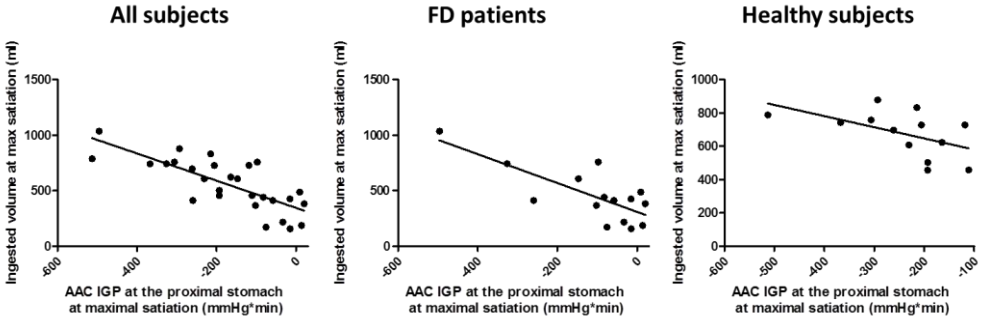


Figure 6. Spearman correlations and linear regression of the AAC of the proximal stomach IGP curve and the maximal ingested volume in all subjects (n=28), in FD patients (n=15) and in healthy subjects (n=13).

The difference in nadir between both groups did not reach significance, probably reflecting the relatively small groups (Nadir HVs: -7±0.6 mmHg vs. FD: -5.4±0.7 mmHg, p=0.13). However, the time to reach nadir IGP was significantly shorter in FD patients (HVs: 6.7±0.8 min and FD: 3.6±0.6 min; p=0.005). The slope of the IGP to the time the 1st patient felt maximally satiated was also not different between groups (HV=-1.76±0.32 mmHg.min^{-1} vs. FD=-1.27±0.4 mmHg.min^{-1};p=0.39).

A correlation between the ingested volume at maximal satiation and nadir IGP in the proximal stomach was observed for all subjects pooled (r=-0.39, p=0.04), but did not reach significance when the groups were analyzed separately (HVs: r=-0.09, p=0.76; FD: r=-0.36, p=0.18).

After the IGP drop, a gradual recovery of IGP back to baseline followed. Relatively to the nadir IGP, the slope from nadir IGP until maximal satiation and the IGP at maximal satiation in FD were similar to HVs (slope FD=0.64±0.2 vs. HV= 0.64±0.2; p=0.99). In all groups, a significant association was found between the recovery of IGP (from nadir) and the increase in satiation scores from nadir IGP (All r= 0.24, HVs r=0.34 and FD r=0.27, p<0.0001).

IGP in the distal stomach

During intragastric infusion of the nutrient meal, IGP in the distal stomach also dropped from baseline in all subjects. In FD patients (n=14), the AAC of the IGP in the distal stomach was similar to the IGP in the proximal stomach at 80% of the measurements in FD (AAC distal: -27.6±7.7 mmHg*min, p=0.3). In the HVs (n=11), the AAC of the IGP in the distal stomach (-25±8.2 mmHg*min) was significantly smaller compared to the AAC at the proximal stomach (p=0.003). Compared to HVs, the parameters of the distal stomach IGP curves until were similar in FD patients (AUC p=0.88, nadir p=0.48). But again, the time to reach nadir in FD was earlier than in HVs (FD 3.5±0.9 vs. 6.3±1; p=0.04).

In FD, compared to the values in the proximal stomach, nadir IGP in the distal stomach was slightly higher than in the proximal stomach (-3.9±0.6 mmHg; p=0.04) but the time to nadir was similar. A greater difference was observed in HVs, with the distal stomach nadir IGP (-3.3±0.7 mmHg) significantly smaller than the proximal stomach nadir IGP (p<0.0001), but the time to reach nadir (5.8±1.0 min) was similar for both curves (p=0.69) (Figure 7).

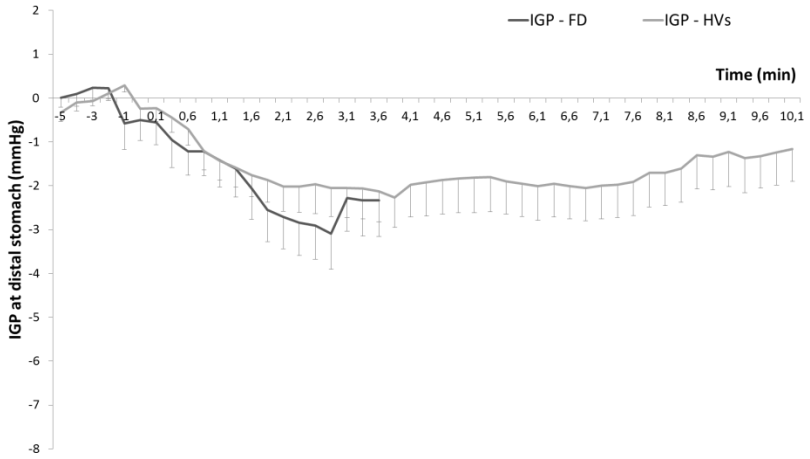


Figure 7. Time course of IGP in the distal stomach during nutrient infusion until maximal satiation in FD patients and healthy subjects.

In contrast to proximal values, no significant associations were found between the AAC of the IGP curve at the distal stomach until maximal satiation with the maximal ingested volume (All subjects r=-0.17, p=0.4; FD r=-0.36, p=0.2; HVs r=0.28, p=0.4) (Figure 8).

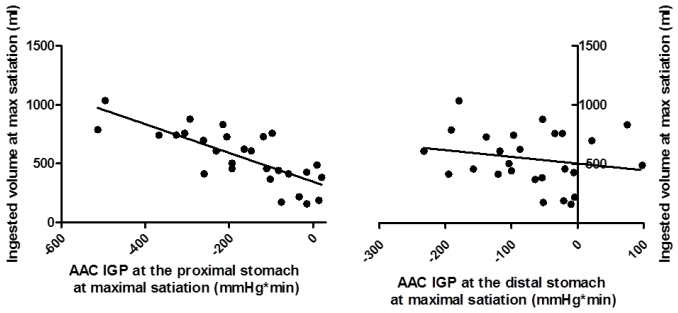


Figure 8. Spearman correlation of the area above the curve (AAC) of the IGP in the proximal and distal stomach in all subjects with the maximal ingested volume at maximal satiation.

From nadir, the distal stomach IGP also increased gradually back towards baseline, although this tended to be slower than the IGP recovery in the proximal stomach (slope distal stomach IGP from nadir FD: 0.40±0.07, p=0.23; HV: 0.33±0.1 mmHg, p=0.16). This gradual increase in IGP from nadir did not differ between FD and HVs (p=0.55).

No significant association was found between the distal stomach nadir IGP and the maximally ingested volume (all subjects r=0.34, p=0.08; FD r=0.08, p=0.76; HV r=0.52, p=0.08). Moreover, similar to the proximal stomach, the rise in distal stomach IGP from nadir was also associated with increasing satiation scores from nadir in all groups (all subjects r=0.23, HV r=0.28, FD r=0.31; p<0.0001) (Figure 9).

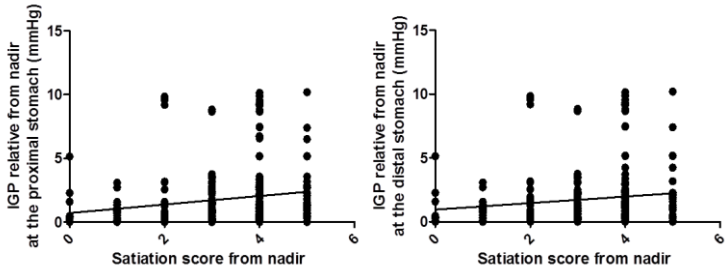


Figure 9. Spearman correlation of the rise in IGP in the distal and proximal stomach from nadir (recovery) and the increasing satiation scores from nadir in all subjects.

Entire IGP measurement (45 min)

The time from the start of the intragastric infusion until the end of the study lasted 45 min in all subjects. After nadir, the IGP increased gradually back to baseline (Figure 10). In healthy subjects the AAC of the IGP in the proximal stomach was significantly higher than the AAC in the distal stomach (-409.7±57.6 vs. -58.7±58.56 mmHg*min; p=0.02). In contrast, in FD patients, the AAC of the proximal stomach and the IGP in the distal stomach during the entire measurement were similar (-203.1±99.6 mmHg*min vs. -87.9±94.7 mmHg, p=0.23). Over the entire measurement, in HVs, the minimal IGP in the proximal stomach was significantly lower than the minimal IGP in the distal stomach (-7.16±0.62 mmHg vs. -3.57±0.77 mmHg, p=0.002). There was no difference in minimal IGP in the distal and proximal stomach in FD patients (-5.64±0.69 vs. -5.26±0.85 mmHg). No difference between patients and HVs was found in the time needed for recovery of IGP back to baseline (data not shown).

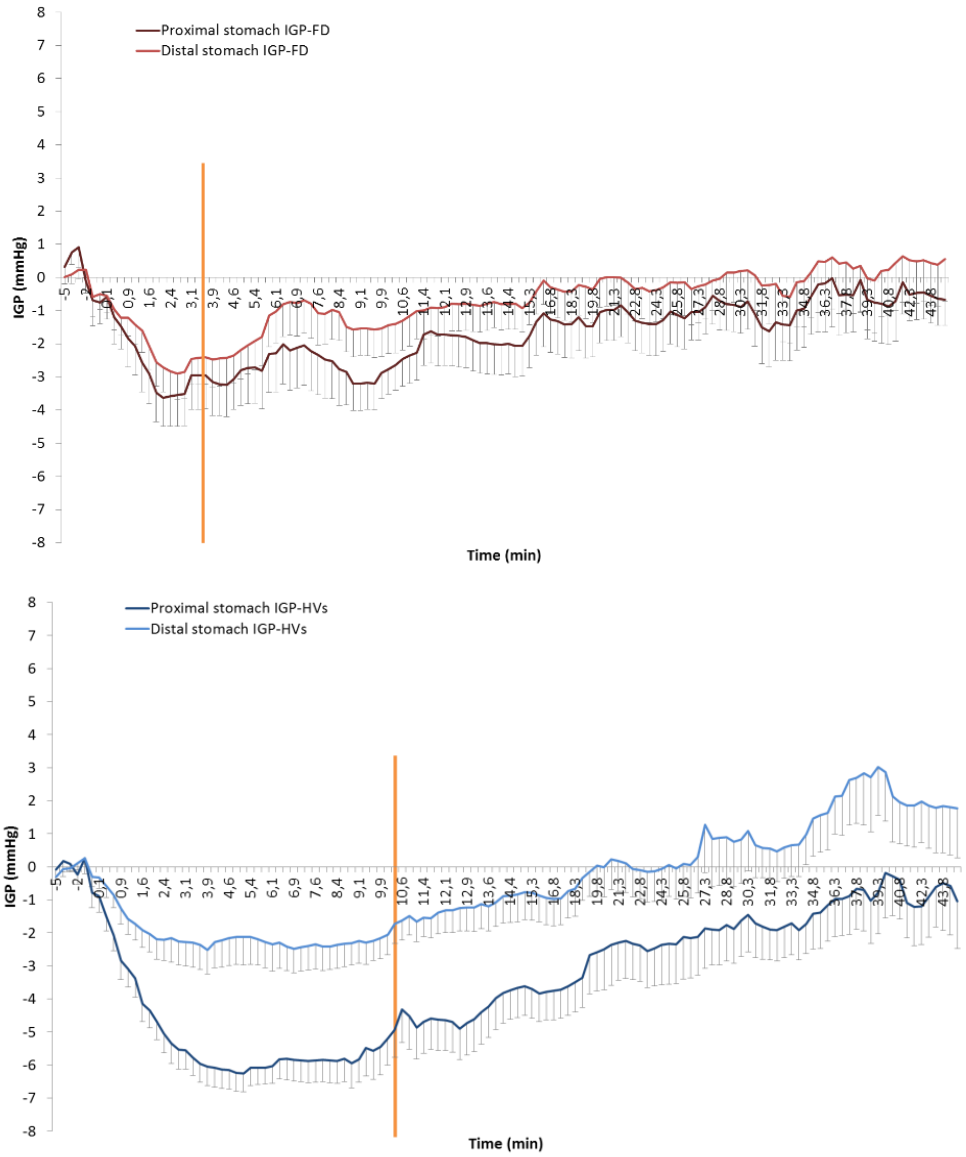


Figure 10. Time course (45 min) of the IGP in the proximal and distal stomach in FD patients (red) and healthy subjects (blue). During intragastric infusion of the nutrient meal, the IGP in the proximal and distal stomach dropped from baseline followed by a gradual recovery back to baseline. The orange line indicates the time point at which 80% of the FD patients (3.6 min) and 80% of the healthy subjects (10.1 min) were still undergoing intragastric nutrient infusion until maximal satiation.

Scintigraphy imaging and intragastric volume distribution

In FD patients as well as in HVs, at the time of reaching maximum satiation, the AUC of the proximal intragastric volume was significantly higher than the value for the distal stomach. However, in comparison to HVs, the volume distribution in the proximal stomach was significantly decreased in FD (AUC HVs 10196±1222 vs. FD 4698±903ml*min, p=0.03). The volume in the distal stomach tended to be increased in patients compared to healthy subjects (AUC HVs 2709±604 vs. FD 4698±837ml*min, p=0.05) (Figure 11). The volume present in the duodenum was also lower in FD compared to HVs (AUC HVs 1.470±300 vs. FD 441±129 ml*min, p=0.003).

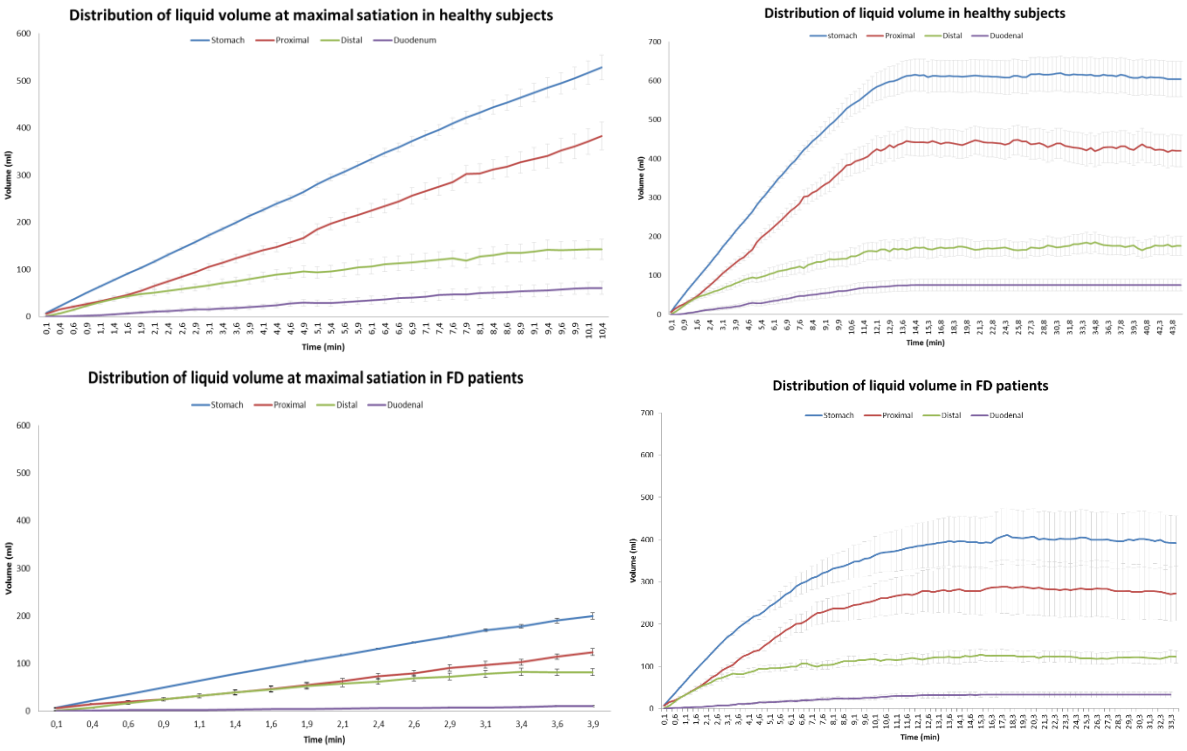


Figure 11. Overview of the time course of proximal and distal filling of the stomach in FD patients and healthy subjects until maximal satiation (left) and over the entire test (right).

The filling of the proximal stomach was better correlated to the satiation scores than the filling of the distal stomach, both in FD and in HVs (Proximal filling and satiation score FD r=0.70; HV r=0.81; Distal filling and satiation score HV r=0.70 p<0.0001; FD r=0.62, all p<0.0001) (Figure 12).
The quantity of fluid emptying to the duodenum during the test until maximal satiation was small. At maximal satiation, volume analysis showed higher residual content in the stomach in FD patients (95±1%) compared to HVs (88±2%; p=0.02).

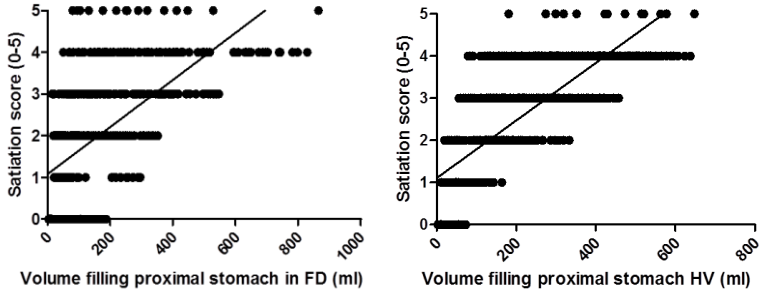


Figure 12. Correlation of proximal filling of the stomach and satiety scores in FD patients and healthy subjects until maximal satiation.

Intragastric pressure and intragastric volume distribution

In all subjects and in FD patients, the AAC of the IGP in the proximal stomach was significantly correlated with the AUC of the scintigraphically determined proximal intragastric nutrient volume until

maximal satiation (ALL r=-0.67, p=0.0001; FD r=0.53; p=0.04). This correlation was not significant in HVs (r=0.27; p=0.37). When AAC of IGP in the proximal stomach was correlated to AUC of the distal intragastric volume, similar results were obtained (all subjects r=-0.68, p<0.001; FD r=-0.64, p=0.01; HV r=-0.65, p=0.02) (Figure 13). The AAC of the IGP in the distal stomach was not correlated to the AUC of proximal or distal volumes in any group (data not shown). Finally, a significant correlation was found between the rise in IGP in the proximal stomach and the rise in proximal filling volume in HVs (r=0.33, p<0.0001), but not in FD (r=-0.18, p=0.005) or in all subjects (r=-0.003,p=0.9).

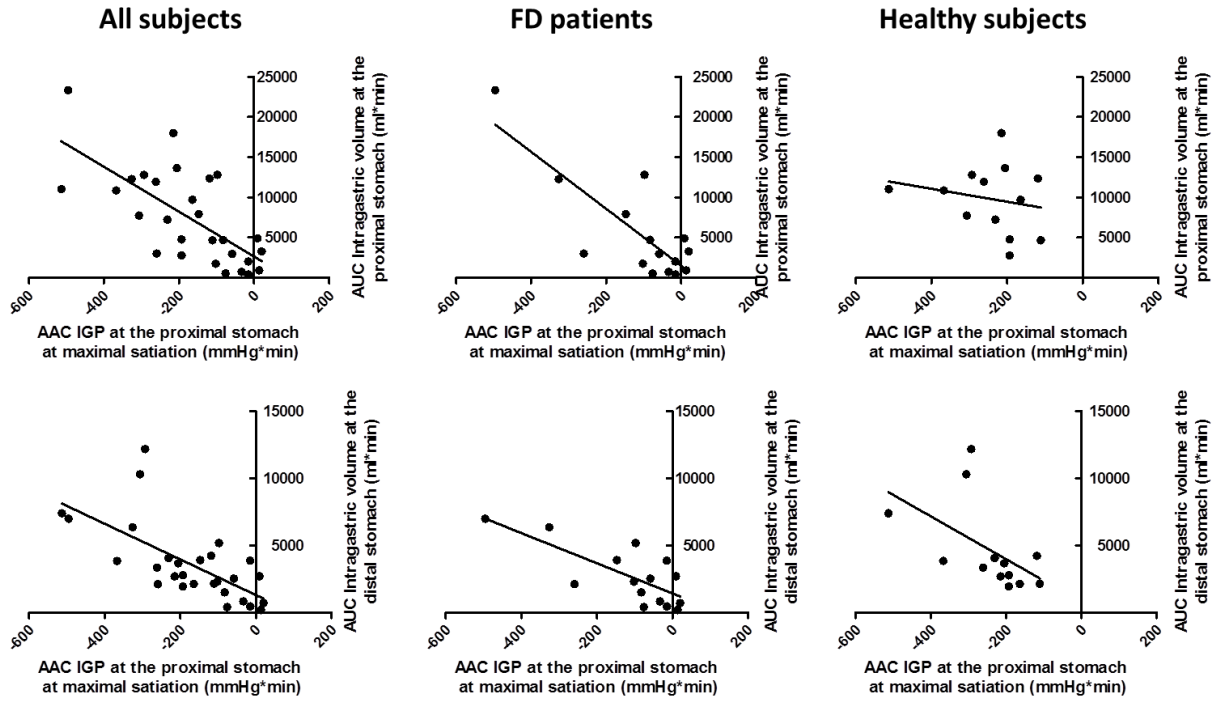


Figure 13: Spearman correlation of the area above the IGP curve in the proximal stomach and the area under the proximal or distal volume curve during scintigraphy.

4.2.4. Discussion

In this study, two methods were used to estimate the effect of gastric accommodation and the relationship between the intragastric pressure and the redistribution of the intragastric content in healthy subjects and FD patients.

The intragastric pressure measurements showed that the drop in IGP in the proximal stomach and the nutrient volume tolerance were decreased in FD patients compared to healthy subjects. In HVs, the drop in IGP in the distal stomach was significantly smaller than in the proximal stomach, indicating that the proximal part of the stomach plays the main relaxatory role during the gastric accommodation reflex. Interestingly, in FD the distal and the proximal stomach IGP drops were similar, indicating that the proximal relaxation is impaired in patients, and that this is not compensated by a bigger distal relaxation. In all subjects as a group, a moderate correlation was found between nadir IGP in the proximal stomach and the amount of ingested volume at maximal satiation, but no significant association was when FD patients and HVs were considered separately. However, variability in these measures in the HV group is small, and the numbers of subjects are low in the subgroups. A similar association was not found when analyzing the IGP in the distal stomach.

After the IGP drop, a gradual recovery of IGP back to baseline follows. The recovery in IGP in the proximal stomach from nadir correlated significantly with the increase in satiation scores and this correlation was highest in healthy subjects. Similar associations were found for the rise in IGP in the distal stomach from nadir. Taken together, these observations point out that a proximal rather than a distal stomach IGP drop is a major determinant of meal-induced satiation, and that impairment in proximal stomach IGP drop is associated with decreased nutrient tolerance. This was also observed in previous studies using IGP measurements during intragastric infusion in healthy subjects (160, 166, 258, 259, 266).

Scintigraphy images showed that the intragastrically infused liquid meal accumulates predominantly in the proximal part of the stomach in HVs. The predominant filling of the proximal stomach during the process of meal-induced satiation rise was suppressed in FD compared to HVs. In HVs, the filling of the proximal stomach correlated better to satiation scores than the distal filling of the stomach. Hence, the imaging data indicate again the importance of the proximal stomach as a reservoir during the gastric accommodation reflex. During the intragastric infusion of the nutrient drink, a small quantity of fluid emptied to the duodenum. The residual gastric content at maximal satiation in FD patients was higher than in healthy subjects, indicating a slight delay in gastric emptying rate in FD, but its impact is yet to be confirmed.

Separately and combined, the methods of IGP and scintigraphy measurements confirm the important function of the proximal stomach as a reservoir for the food and as a determinant of satiation during and after the ingestion of a meal. Both the IGP measurements in the proximal stomach (IGP rise from nadir) and the increase in proximal intragastric volume (filling of the proximal stomach) assessed by scintigraphy were significantly correlated with the rise in meal-induced satiation scores. Only the filling of the proximal stomach correlated well with the rise in IGP in the proximal stomach from nadir in HVs. Furthermore, the area above the IGP in the proximal stomach curve was significantly correlated to the filling of the proximal and the distal stomach until maximal satiation in all subjects and in the patients, but not in the group of healthy subjects alone. No association was found between the AAC of the IGP in the distal stomach and the proximal and distal volumes. Finally, an association was found in healthy subjects with proximal nadir IGP and the proportion of nutrient present in the proximal stomach. This was not observed with the distal stomach values.

A decreased gastric accommodation is a pathophysiological mechanism recognized in at least a subset of functional dyspepsia patients. It has already been documented that impairment during gastric accommodation might cause symptoms such as early satiation, postprandial discomfort and the longer-term consequence of unintentional weight loss (22). Previous studies with the barostat and slow drinking test used NG-monomethyl-L-Arginine (L-NMMA), a NO-synthase inhibitor, to induce impaired gastric accommodation. These studies showed reduced proximal gastric relaxation in association with decreased nutrient tolerance (9, 36). In a similar study design in HVs, IGP measurements during the intragastric infusion after infusion of L-NMMA showed a suppression of the IGP drop and this was associated with decreased nutrient tolerance, indicating that a decreased IGP drop corresponds to an impaired gastric accommodation (160). The present study showed that FD patients have a decreased IGP drop and nutrient tolerance compared to healthy subjects, implying the impairment on their gastric accommodation during the intragastric infusion of the liquid meal. Previous scintigraphy measurements used to estimate gastric accommodation showed, as in our study, that that after ingestion of a radiolabeled meal most of the marker resided in the proximal part of the

stomach, and redistributed to the distal stomach after a period of time (79). Compared to healthy subjects, and similar to the observation by Piessevaux et al. (195), scintigraphy images in FD showed that the accumulation of the meal in the proximal was decreased, with more accumulation in the distal stomach (79). This was correlated to the decreased IGP drop and nutrient tolerance in patients. In the study of Piessevaux et al., increased distal accumulation of the meal was associated with more prevalent symptoms of early satiation, while another subset of FD patients showed increased proximal retention of the meal and this was associated with increased prevalence of postprandial fullness (195). From these and the present data, it can be concluded that abnormal distribution of the meal to the distal stomach may be the result of impaired gastric accommodation, which can be quantified as a decreased IGP drop during nutrient infusion.

In the present study more than 80% of the meal was still retained in the stomach during and until the end of the intragastric infusion of the liquid nutrient meal indicating that the intragastric content remained rather constant after some initial emptying to the duodenum. This observation facilitated the interpretation of the results that implicate the changes in IGP as an important role player for the distribution of the intragastric content.

The small number of subjects and the significant young age of controls compared to the FD patients are important limitations in our study design and they should be taken into account when interpreting the results.

Overall, these results indicate that the IGP measurements correlate well with the reservoir function of GA in the proximal stomach and nutrient tolerance. The IGP technique is less invasive and easier to perform than the gastric barostat, potentially allowing its use in advanced clinical practice in different patient populations, including pediatrics (160, 258, 261, 266). As the IGP probe not only measures the IGP of the proximal stomach, but also the activity of the low esophageal sphincter and the distal stomach, its potential for application in studies of the (patho-) physiology of the entire upper gastrointestinal tract is broad. The technique also holds major promises for new drug development in FD and other food intolerance- or motility-like disorders.

In conclusion, the IGP measurement is a valid tool to measure the gastric accommodation reflex in FD patients and HVs. During the intragastric infusion of the meal, the filling of the proximal stomach is the main determinant of meal-induced satiation. This is associated to a drop of the proximal intragastric pressure and a gradual recovery of IGP after nadir back to baseline. Impaired gastric accommodation with decreased nutrient tolerance is associated to a decreased IGP drop in the proximal stomach and impaired accumulation of the intragastric content in the proximal stomach, with redistribution to the distal stomach.

Chapter 5

Evaluation of novel therapeutic pathways with the gastric barostat and intragastric pressure measurement.

5.1.1. Introduction

Upon food intake, gastric distension activates mechanosensitive receptors in the gastric wall that send signals through a vago-vagal reflex pathway to induce relaxation of the proximal stomach (160, 267). This pathway, called the accommodation reflex, facilitates temporary storage of ingested food without a rise in intragastric pressure (IGP). Relaxation of smooth muscle in the proximal stomach is mediated through release of NO from non-adrenergic non-cholinergic (NANC) inhibitory neurons in the gastric wall (36, 267). NO diffuses through the cell membrane of the smooth muscle cells where it increases the concentration of cyclic guanylyl monophosphate (cGMP) and this initiates a process that ends in hyperpolarization. As a result, the smooth muscles of the proximal stomach relax to keep IGP low while the intragastric volume increases (268). Progressive filling of the proximal stomach induces a rise in IGP, accompanied by the feeling of satiation and followed by redistribution of gastric content from the proximal stomach to the antrum, allowing the initiation of gastric emptying. The tissue levels of cGMP are determined and balanced by guanylyl cyclase (GC) and cGMP-specific phosphodiesterase-5 (PDE5). The former catalyses cGMP formation, while the latter induces its degradation by hydrolysation (268).

Sildenafil citrate is a potent specific PDE5 inhibitor which is used in the treatment of erectile dysfunction (269, 270). Moreover, during sildenafil clinical trials and post-clinical studies, it has been reported that sildenafil induces dyspeptic symptoms as the most frequent adverse event besides headache and flushing (269-271). Therefore, it is conceivable that sildenafil might have an effect on gastric motility. Earlier studies in healthy volunteers reported that sildenafil enhances meal-induced accommodation as measured by a gastric barostat, and does not alter solid emptying but significantly delays liquid gastric emptying (272). However, the presence of a balloon in the stomach could disrupt the normal physiologic responses to food intake and therefore, it might exaggerate the natural responses of GA and GE (158, 160). More recently, IGP measurement during nutrient intake was developed as a more physiological method to measure GA (160). Our aim was to evaluate the effect of sildenafil on meal-induced satiation and GA during nutrient drink ingestion, measured with this minimally invasive and potentially more accurate alternative for the barostat. In addition, we used the C^{13}-breath test to quantify GE.

5.1.2. Materials and Methods

Study subjects

All study procedures were approved by the Ethics Committee of Leuven University Hospital, Belgium. Twenty healthy volunteers (HVs) participated in this single-blind cross-over study. The exclusion criteria included the presence of symptoms or a history of gastrointestinal diseases, any other significant disease or psychological disorder and pregnancy, or the use of any medication that may affect gastric sensorimotor function. HVs were asked to come to the clinic after fasting overnight. They were asked to refrain from alcohol, tea and coffee for at least 12 hours before participation, moreover they were asked to refrain from smoking cigarettes at least 1 hour before the start of the experiments. A written informed consent was obtained from each participant.

IGP measurement

IGP was measured by means of a HRM catheter, as previously described (160, 258, 273, 274). A manometry probe (ManoScan 360, Sierra Scientific instruments, Los Angeles (USA)), a small, flexible tube, was passed through the nose into the stomach. The probe contains 36 channels that measure pressure. The manometry probe was positioned in the stomach of the volunteer. Its position was then verified by fluoroscopy. To infuse the nutrient drink directly into the stomach, a second infusion catheter (Nutricia Flocare line, Bornem, Belgium) was positioned through the mouth of the volunteers and advanced until the tip of this infusion catheter was located in the proximal stomach.

The catheters were fixed to the subjects' chin and the volunteers were asked to take place in a bed in a comfortable sitting position with the trunk upright. After a stabilization period, an oral dose of sildenafil (50 mg, Viagra®, Pfizer, UK) in an opaque gel capsule or a placebo (opaque gel capsule) was administered to the volunteers in a randomized fashion. The gel capsule was used to hide the colour and shape of the Viagra® pill from the volunteers. Forty-five minutes hereafter, a nutrient drink (Nutridrink®, Nutricia, 150 kcal per 100 ml with 6 g proteins, 18.4 g carbohydrates and 5.8 g lipids, Netherlands) was infused directly into the stomach of the volunteer at a constant speed of 60 ml/min.

During the study, volunteers were asked to fill out visual analogue scales (VAS) for hunger, satiation and 6 epigastric symptoms (fullness, nausea, belching of air, cramps in the abdomen, bloating and pain) at 5-minute intervals. In addition, during nutrient drink infusion they also had to score their satiation at 1-minute intervals by using a graphic rating scale that combines verbal descriptors on a scale graded from 0–5 (1, threshold; 5, maximum satiation).

Intragastric infusion was stopped as soon as the volunteers reached the maximum score of 5 on their satiation scale or when they score maximally on one of the epigastric symptoms. Five minutes hereafter the catheters were disconnected and removed and the volunteers could leave the hospital (Figure 1).

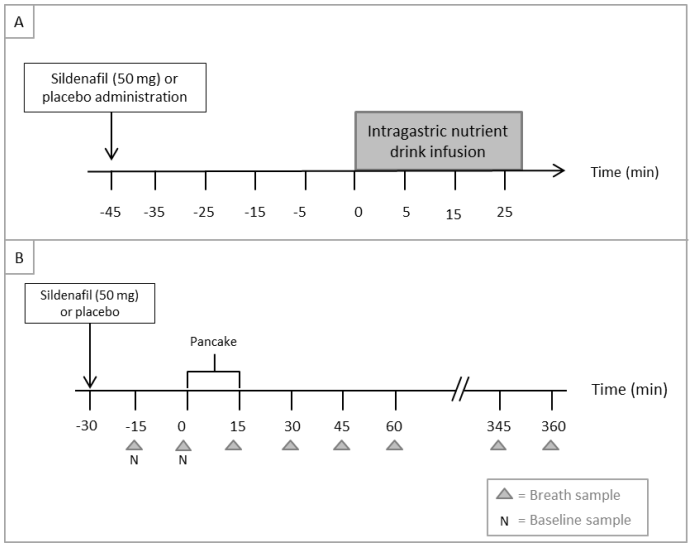


Figure 1: A. Representation of the IGP protocol on a time scheme in minutes. Sildenafil or placebo was administered 45 minutes before nutrient drink infusion. Intragastric infusion was stopped when the volunteers reached the maximum score on their satiation scale. B. Representation of the breath test protocol on a time scheme in minutes. Between ingestion of the drug and the pancakes, volunteers had to give two neutral (N) breath samples. After the pancakes were ingested, breath samples were given every 15 minutes until 6 hours postprandial.

Gastric emptying measurement

The C^{13}-breath test was used to measure gastric emptying rate. After an overnight fast, volunteers ingested a standardized solid meal consisting of one non-radioactive ^{13}C-octanoic acid labelled pancake (244 kcal) within 15 minutes. Sildenafil (50 mg, Viagra®) or placebo was administered to the HVs in a randomized fashion and two control breath samples were given. Thirty minutes hereafter the pancake was ingested. For consumption of the pancake, 5 g sugar was added as a sweetener and water was given as a drink. After eating, volunteers gave a breath sample and scored their satiation every 15 minutes until 6 hours postprandial. The breath samples were collected in sample tubes and GE rate was analysed by determining the exhaled $^{13}CO_2$/ $^{12}CO_2$ ratio .

Data analysis IGP measurements

The IGP original data was imported from the recorder software ManoAcquisition® to Excel. The data was calculated as previously described by Jansen et al. The IGP was measured as the average pressure of the first five pressure channels that were clearly positioned below the lower esophageal sphincter or the pressure area influenced by the LES. To avoid influence from movements such as swallowing, moving, etc., a moving median was calculated from the original data (median value over 1 minute of the original data). Per channel, a baseline value was calculated from the moving median data corresponding to the minimum pressure in the last 5 minutes of the stabilization period before nutrient drink infusion. The paired t-test was used to compare the mean AUC between IGP curve of the sildenafil and placebo groups.

The nadir IGP was defined as the minimal IGP or the lowest relaxation point during nutrient drink infusion. Mean AUC satiation scores curves and the mean volume and time to reach maximal satiation were compared with the paired t-test. The slope of the satiety score curve was calculated by linear interpolation and compared between groups with the paired t-test.

The visual analogue scales for epigastric symptoms (fullness, nausea, belching of air, cramps in the abdomen, bloating and pain) at 5-minute intervals for the sildenafil group and placebo group were measured and the mean AUC was compared with the paired t-test. In all analyses $p < 0.05$ was considered significant. All data was presented as mean ± SEM.

Data analysis gastric emptying measurements

Isotope Ratio Mass Spectrometry measured the abundance profile of ^{13}C and compared it to the abundance of ^{12}C in the sample tubes. Then, the ratio of $^{13}C/^{12}C$ was calculated and compared to a conventional reference of ^{12}C abundance. Data were then imported to Excel where the $T_{1/2}$ (time when 50% of the meal had emptied from the stomach) was automatically calculated. In this calculations the molar mass of the substrate and its dose were taken into account. Student's paired t-test was used to compare the means of $T_{1/2}$ between placebo and sildenafil groups. In all analysis $p < 0.05$ was considered significant. All data was presented as mean ± SEM.

5.1.3. Results

Conduct of the study

All 16 HVs (mean age: 30.1±3.2 years old, mean BMI: 23.2±0.5 kg/m^2, Female: 68%) completed the study as planned. All procedures were well-tolerated and no adverse events occurred.

IGP during nutrient infusion

GA was initiated when nutrient drink infusion started. In an initial phase, IGP progressively decreased from baseline pressure, followed by a phase during which IGP stabilized and recovered until maximal satiation (Figure 2). After placebo treatment, the infusion of nutrient drink caused an IGP drop to a nadir of -6.7±0.9 mmHg. After sildenafil treatment, the IGP dropped from baseline to a nadir of -4.3±0.9 mmHg. However, the nadir was not significantly different compared to placebo (p=0.06). Thereafter, IGP gradually increased until the end of the experiment (Figure 2).

The average AUC of the IGP curve during nutrient drink infusion until maximal satiation was significantly lower after sildenafil treatment compared to placebo (-33.6±8.8 vs. -60.8±11.3 mmHg; p=0.005).

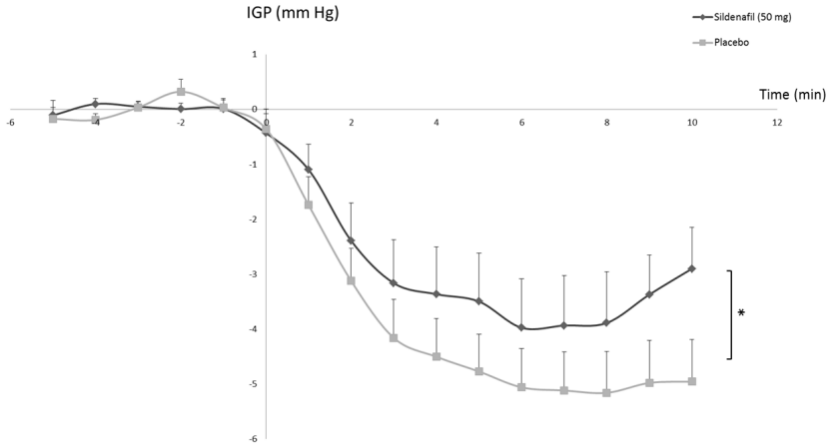


Figure 2: Intragastric pressure change over time. During both treatments the IGP progressively decreased from baseline pressure when nutrient drink infusion was started. During the control study the IGP stabilized after 5 minutes. However, during sildenafil study, the IGP first drop was less than during placebo and then it gradually increased until the end of the experiment. The results were presented as mean ± SEM.

Effect of sildenafil on satiation

Sildenafil treated volunteers scored maximal satiation at a significantly lower volume compared to placebo treated subjects (678.8±70.1 mL vs. 836.3±82.6 mL; p=0.02) (Figure 3). The satiation score curve increased in a quasi-linear fashion during nutrient infusion. After sildenafil, compared to placebo the average AUC of the satiation curve was significantly higher (28.6±2.3 vs. 34.2±2.9 min*satiation units; p=0.04). Moreover, the linear slopes of the satiety scores curves were significantly different between the groups (0.4±0.02 and 0.3±0.03 min^{-1}, sildenafil and placebo respectively, p=0.04). Dyspeptic symptom intensity assessed by VAS scores did not differ significantly between both groups.

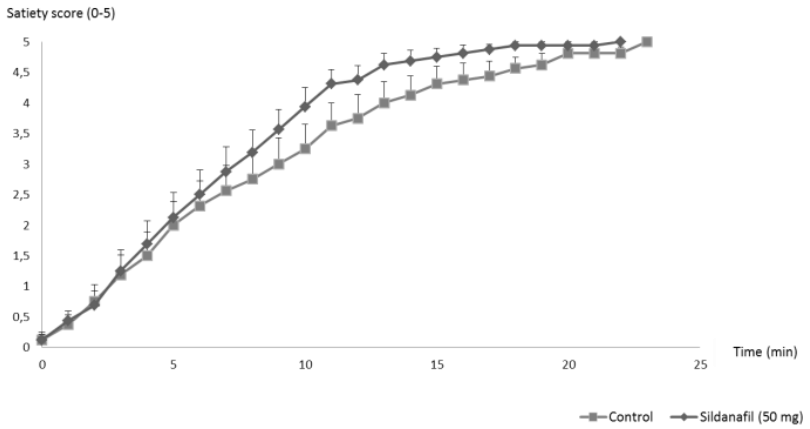


Figure 3: Nutrient challenge test. Left, HVs (n=16) drank significantly less after oral sildenafil treatment (50 mg) compared to placebo (p=0.019). HVs drank 836 ± 82.6 mL after placebo and 678 ± 70 mL after sildenafil. Average values were compared with the student's T-test. The results were presented as mean ± SEM.

Gastric emptying rate

After sildenafil, the half gastric emptying time ($T_{1/2}$) was significantly slower compared to placebo ($T_{1/2}$: 76.6±7.1 minutes for placebo vs. 90.6±5.9 minutes for sildenafil; p=0.04)

5.1.4. Discussion

Sildenafil citrate is a potent specific PDE5 inhibitor which is used in the treatment of erectile dysfunction (269-271). During sildenafil clinical trials and post-clinical studies, it has been reported that sildenafil is mostly well tolerated, although it may induce some side effects such as headache, flushing and dyspeptic symptoms (269-272). The mechanism of action through which sildenafil induces dyspeptic symptoms is unknown. Therefore, in this study we focused on the effects of sildenafil on the gastric motor function.

It has previously been described by barostat and HRM experiments that IGP decreases during stomach distention triggered by a meal; therefore IGP could be used to asses gastric accommodation (160). Moreover, by means of the HRM IGP measurements during nutrient drink infusion in the stomach, an association between IGP and satiation during meal ingestion can be observed (160, 258, 273, 274). Several studies have used IGP measurement to describe gastric motility and control of satiation in healthy volunteers, supporting the concept that the IGP drop reflects gastric accommodation (160, 258, 273, 274). Moreover, in this studies it was observed that the HRM was less invasive and easier to perform than the gastric barostat when measuring overall gastric activity in healthy volunteers (160, 258, 273, 274).

In the present study, the IGP was measured by the HRM to estimate changes in gastric muscle tone after oral administration of 50 mg sildenafil in healthy volunteers. It was observed that oral administration of sildenafil significantly suppressed the drop in IGP during nutrient infusion, suggesting an inhibitory effect of sildenafil on GA. In addition, we observed that the healthy subjects reached earlier maximal satiation after sildenafil treatment and consequently ingested significantly less nutrient volume. These nutrient tolerance effects are in agreement with a possible underlying decreased GA. Furthermore, the GE rate for solids was delayed by the same sildenafil dose. Literature reports on the effect of sildenafil on gastric emptying rate show divergent outcomes, and both

absence of a significant effect on gastric emptying and delay in emptying after sildenafil treatment have been reported (272, 275, 276). It is conceivable that our observations explain the occurrence of dyspeptic symptoms after sildenafil intake: impaired GA and delayed GE are well-established pathophysiological mechanisms in FD and are associated with postprandial fullness, nausea, vomiting, decreased nutrient tolerance, early satiation and weight loss (21, 22, 73).

The finding of an inhibitory effect of sildenafil on GA was unexpected: sildenafil would be estimated to enhance the relaxatory effect of NO, considering its mechanism of action described in earlier animal and human studies (8, 36, 160, 277, 278). However, studies over the last years have established that the gastric accommodation reflex is not mediated by a single mediator such as NO, but that it is a complex phenomenon in which a range of neurotransmitters are involved such as serotonin, endogenous opioids and endocannabinoids (23, 32, 279). Moreover, factors determining the size of GA are not only the arrival of nutrients in the stomach, but also feedback from the duodenum and intragastric antro-fundic reflex pathways (20, 33, 80).

Paradoxically, a previous gastric barostat study in fact showed enhanced postprandial gastric volumes in response to sildenafil administration, suggestive of enhanced GA (272). In contrast, the current study, using IGP monitoring, suggested inhibition of GA by sildenafil. The results of the satiation test are in line with an inhibition of gastric volume capacity by sildenafil, and thus support the concept of impaired GA (163). Two factors may play a role in these differences. First of all, the gastric barostat exerts a positive distending force on the proximal stomach, and this may artificially increase the proximal stomach volume as a consequence of reflex-driven changes in gastric tone (158, 257). It is conceivable that sildenafil alters some of the reflex pathways that are driven by the distending force of the balloon in the proximal stomach. Second, while IGP measurement provides information on pressure events in different regions of the stomach (36 measurement points), the barostat balloon extends from the proximal stomach into the distal stomach and may be influenced by events in the antrum (158, 257). In man, proximal and distal gastric motor activity are closely correlated, and both the proximal and distal stomach relax during nutrient drink infusion (280, 281). Previously, Bortolotti reported that sildenafil inhibited phasic contractions in the antrum as well as the duodenum (282), and Cho et al. found evidence for rapid redistribution of radiopaque markers from the proximal stomach to the distal stomach (275). Hence, it is conceivable that sildenafil primarily inhibits antral tone and contractility, leading to redistribution of the gastric content from the proximal to the distal stomach and, therefore resulting in a more restricted gastric relaxation, and consequently IGP drop, at the level of the fundus. Antral hypocontractility and distention is likely to result in delayed gastric emptying, and this is consistent with the effects we found with the gastric emptying breath test in the current study. Furthermore, as the distal stomach is less compliant, antral distention is more likely to induce dyspeptic symptoms, and this may contribute significantly to the induction of dyspeptic symptoms after sildenafil (280, 281). Providing solid proof for redistribution of the meal to the antrum after sildenafil would require additional imaging studies and is beyond the scope of the current protocol.

In conclusion, in man 50 mg sildenafil inhibits the IGP-drop of the proximal stomach upon nutrient ingestion, suggestive of impaired gastric accommodation. This was associated with significantly decreased nutrient tolerance and delayed solid gastric emptying in healthy volunteers. These observations may underlie occurrence of dyspeptic symptoms induced by sildenafil.

5.2.1. Introduction

The Rome III criteria defined functional dyspepsia (FD) as "the presence of chronic dyspeptic symptoms in the absence of any structural or metabolic disease that is likely to explain the symptoms" (41, 42). FD is one of the most common functional gastrointestinal disorders in clinical practice with an estimated population prevalence of 5-15% (41-43, 196). The heterogeneous nature of the disease and the lack of established effective therapeutic options underlie the high socioeconomic and quality of life impact of the condition (42, 195, 196).

In 2006, the Rome III consensus proposed to subdivide FD into Postprandial Distress Syndrome (PDS), characterized by meal-related symptoms such as early satiety and postprandial fullness, and Epigastric Pain Syndrome (EPS) characterized by epigastric burning and epigastric pain, in order to facilitate the diagnostic and therapeutic approach to FD patients (42, 46, 57). Mainly in PDS patients, disorders of gastric sensorimotor function, such as impaired accommodation, delayed gastric emptying and hypersensitivity to gastric distention, have been implicated in symptom generation (16, 22, 58, 62, 78, 189-191). Based on this assumption, prokinetics are recommended as initial therapeutic approach for PDS patients (96, 222).

Agonists at serotonin (5-HT) type 4 receptors are probably the best studied prokinetic agents for upper gastrointestinal disorders. Prucalopride, a highly selective 5-HT_4 receptor agonist, is approved for the treatment of chronic constipation in patients with insufficient response to laxatives (283-287). After oral administration, prucalopride is well absorbed in the gastrointestinal tract and it has an absolute bio-ability of more than 90% (287). The plasma half-life of prucalopride (2 mg) is 24 hours and it reaches the maximum serum concentration between 2 and 3 hours after intake (284, 287). Furthermore, prucalopride has shown a favorable safety profile in studies and clinical practice and it does not affect the QT interval (115, 287). Prucalopride stimulates colonic transit, and this is the basis for its effectiveness in chronic constipation (283, 284, 288, 289). Prucalopride also affects gastric motility as it was shown to enhance gastric emptying in a dog model, in healthy volunteers and in patients with chronic constipation (283, 284, 290). While these gastroprokinetic effects of prucalopride suggest a potential for application in the treatment of motility disorders of the upper gastrointestinal tract, it is relevant to assess the effects of the drug on other aspects of upper gastrointestinal sensorimotor function. Indeed, it has been argued that the lack of symptomatic benefit in upper gastrointestinal motility disorders with prokinetic agents of the motilin class was attributable to their adverse effects on gastric accommodation and gastric sensitivity to distention, which induced early satiation and increased postprandial discomfort (17, 127, 291, 292). Hence, the aim of this study was to investigate the effect of prucalopride on gastric accommodation, sensitivity to gastric distention and nutrient tolerance in healthy subjects.

5.2.2. Materials and Methods

Subjects and study design

Healthy volunteers (HVs), recruited by public advertisement, were invited to participate in a single blind randomized cross-over study with prucalopride (2 mg, Resolor®, Shire, Belgium) and placebo. The study consisted of two parts: a placebo-controlled cross-over gastric barostat study and a placebo-controlled cross-over intragastric pressure (IGP) measurement study. HVs had to be devoid of GI

symptoms and of the use of medications known to influence the GI motility. The study was approved by the Ethics Committee of the University Hospitals, Leuven, Belgium and informed consent was obtained from all subjects before the start of the study.

Gastric barostat study

Following an overnight fast of at least 12 hs, a double lumen polyvinyl tube (Salem sump tube 14 Ch., Sherwood Medical, Petit Rechain, Belgium) with an adherent plastic bag (1200 mL capacity) which was finely folded, was introduced through the mouth and secured to the subject's chin with adhesive tape. The HVs were then asked to take placed in a specifically designed bed in a sitting position with the knees slightly bent. The polyvinyl tube was connected to a computer-driven programmable volume-displacement barostat device (G&J Electronics Inc., Toronto, ON, Canada). To unfold the intragastric bag, it was inflated with a fixed volume of 300 mL of air for 2 min and again deflated completely.

After a 10 min equilibration period, the minimal distending pressure (MDP) was determined by increasing the intrabag pressure by 1 mmHg every minute until the intrabag volume of 30 mL or more was stable for 2 minutes (Figure 1.A).

For the evaluation of the gastric sensitivity and compliance, stepwise isobaric distentions (increments of 2 mmHg every 2 minutes starting from the MDP) were initiated. Distentions were performed before treatment (fasted state), 2 hs after study drug intake (estimated maximal plasma concentration of prucalopride) and after the meal challenge (postprandial state) (Figure 1.A). At every distending step, the subjects were instructed to rate the intensity of upper abdominal sensation (0: no sensation – 5: discomfort and 6: epigastric pain) induced by every stimulus. The procedure concluded when subjects reported maximal discomfort or pain (score 5-6) or when the intrabag volume reached 1000 mL.

For the meal challenge the pressure level was set at MDP + 2 mmHg. After a 30 min baseline period, HVs ingested 200 ml of a nutrient liquid meal (Fortimel Energy®, Nutricia, 150 kcal per 100 ml with 5.9 g proteins, 18.4 g carbohydrates and 5.8 g lipids, Netherlands). Measurement continued for 60 minutes postprandial, when the distention series was repeated (Figure 1.A). During the entire accommodation study, volunteers were asked to fill out visual analogue scales (VAS) for hunger, satiation and 6 epigastric symptoms (fullness, nausea, belching of air, cramps in the abdomen, bloating and pain) at 5-minute intervals (Figure 1.A).

Intragastric pressure measurement study

A high resolution manometry probe (HRM, 36 pressure measurement points, ManoScan 360, Sierra Scientific instruments, Los Angeles (USA)) was passed through the nose into the distal stomach of the HVs. To infuse the nutrient drink directly into the stomach, a second nasogastric feeding tube (Enteral™, Maxter-catheters, Marseille, France) was positioned through the nose into the proximal stomach (160). The position of the catheters was verified briefly by fluoroscopy. The catheters were fixed to the subjects' nose and the subjects were asked to take place in a bed in a comfortable sitting position with the knees slightly bent.

After a stabilization period of 10 minutes, an oral dose of prucalopride (2 mg) or placebo was administered to the volunteers in a randomized fashion. Two hours hereafter, a nutrient drink (Fortimel Energy®, Nutricia, 150 kcal per 100 ml with 5.9 g proteins, 18.4 g carbohydrates and 5.8 g lipids, Netherlands) was infused directly into the stomach at a constant speed of 60 ml/min.

During the study, volunteers were asked to fill out visual analogue scales (VAS) for hunger, satiation and 6 epigastric symptoms (fullness, nausea, belching of air, cramps in the abdomen, bloating and pain) at 5-minute intervals. In addition, during nutrient drink infusion they also scored the level of satiation at 1-minute intervals by using a graphic rating scale that combines verbal descriptors on a scale graded from 0–5 (5, maximum satiation).
Intragastric infusion was stopped as soon as the volunteers reached the maximum score of 5 on their satiation scale or when they score maximally on one of the epigastric symptoms. Five minutes hereafter the catheters were disconnected and removed and the volunteers could leave the hospital (Figure 1.B).

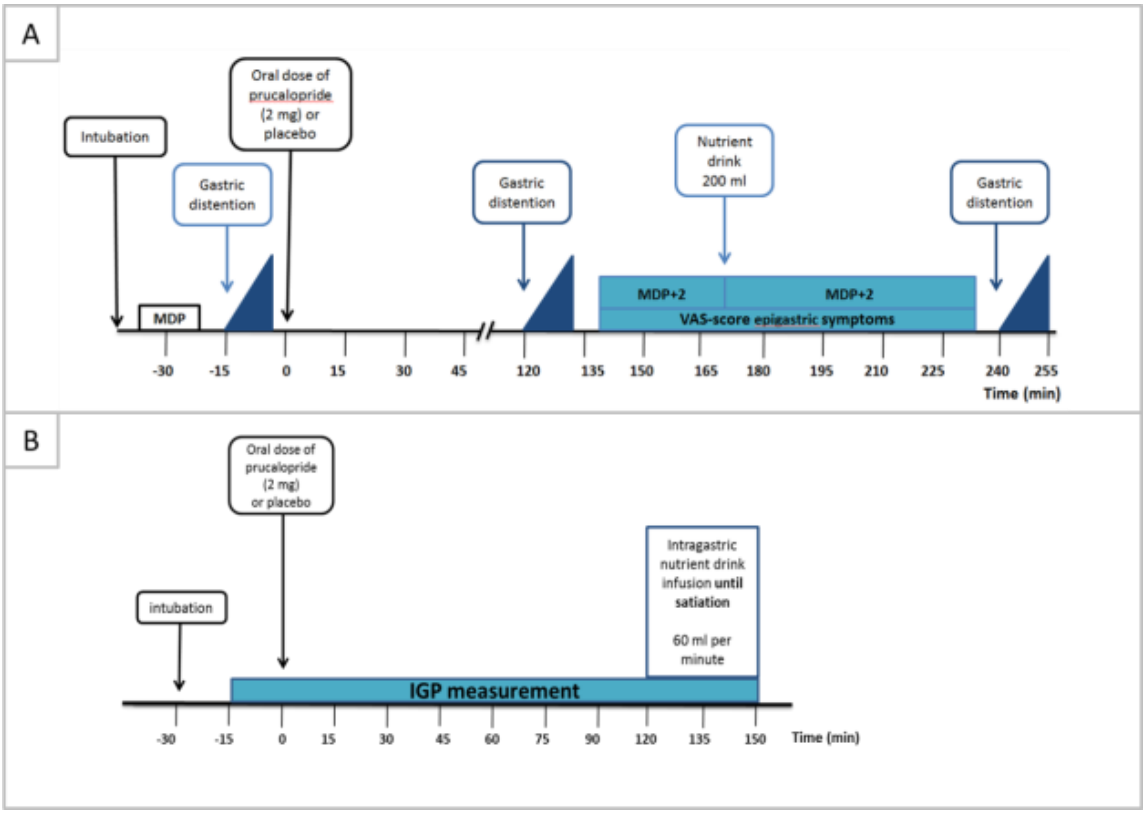


Figure 1: Study design. A. Time line of the gastric barostat measurement. B. Time line of the intragastric pressure measurement

Data Analysis

Gastric barostat study

In the gastric sensitivity studies, for each 2 min distending period, the mean intragastric volume was calculated. The perception threshold was defined as the first level of pressure and the corresponding volume that evoked a perception score of 1 or more. Discomfort threshold was defined as the first level of pressure and the corresponding volume that provoked a sensation score of five or more. The gastric compliance of the subjects was calculated as the slope of the volume/pressure curve. The gastric sensitivity to distention of the subjects was calculated as the slope of the sensitivity scores/pressure curve.
Gastric tone before and after administration of the meal was measured by the calculation of the mean balloon volume for consecutive 5-min intervals. Fasting volume (baseline) was measured as the mean

volume of the 30 min period before ingestion of nutrient drink and the mean postprandial volume was defined as the mean volume one hour after ingestion of the nutrient drink (200 ml). Measurements were done by using commercially available software (Protocol plus™ and Protocol plus™ data scanner). The meal-induced accommodation response was determined as the difference (delta) between the mean volume before (30 min) and after meal intake (60 min). Gastric tone was calculated for the 30 min before the meal, 15 min after the meal and 60 min after the meal.

Gastric motility index (MI), previously defined by (293) as the area between the signal and the baseline normalized over time, was calculated before and after meal intake as a measure for the phasic gastric motility. The baseline reconstruction was performed by analyzing the phasic contractility of the stomach which corresponds to slow changes in baseline volume after filtering out (respiratory) artifacts.

The visual analogue scales (0-100 mm) for upper abdominal symptoms (fullness, nausea, belching of air, cramps in the abdomen, bloating and pain) at 5-minute intervals for the prucalopride group and placebo group were compared by Chi square test of pre- and postprandial area under the curve (AUC) of the symptoms scores over time (10 minutes before and 10 min after the meal).

In all analyses P-value of <0.05 was considered statistically significant. Calculations were compared for treatment versus placebo by paired ***t***-test and correlations were done with Spearman's rank correlation coefficient. All data are presented as mean ± SEM.

Intragastric pressure measurement study

The IGP original data were imported from the recorder software ManoAcquisition® to Microsoft Excel. The data were calculated as previously described by Jansen *et al* (160). The proximal IGP was measured as the average pressure of the first five pressure channels that were clearly positioned below the lower esophageal sphincter (LES) or the pressure area influenced by the LES. The distal IGP was measured as the as the average pressure five pressure channels that were clearly positioned in the distal part of the stomach, characterized by antral contractions during the fasted state.

To avoid influence from movements such as swallowing, moving, etc., a moving median was calculated from the original data (median value over 1 minute of the original data). Per channel, a baseline value was calculated from the moving median data corresponding to the minimum pressure in the last 5 minutes of the stabilization period before nutrient drink infusion. The paired t-test was used to compare the mean area above the curve (AAC) between the IGP curves of the prucalopride and placebo groups. The nadir IGP was defined as the minimal IGP or the point of maximal relaxation during nutrient drink infusion. Mean AUC of the satiation score curves and the mean volume and time to reach maximal satiation were compared with the paired t-test. The slope of the satiety score curve was calculated by linear interpolation and compared between groups with the paired t-test.

Prior the meal, motility index was calculated as previously described by (294, 295). The calculation of mean motility index from 6 antral channels was based on the number of contractions × average amplitude contractions × average duration contractions divided by 5 minutes.

The visual analogue scales for epigastric symptoms (fullness, nausea, belching of air, cramps in the abdomen, bloating and pain) at 5-minute intervals for the prucalopride group and placebo group were compared with the paired t-test.

In all analyses $p < 0.05$ was considered significant. All data are presented as mean ± SEM.

5.2.3. Results

Study subjects demographics

A total of 17 healthy subjects (59% females, mean age 29.4±2.7 years, BMI 22.5±0.5 Kg/m2) were enrolled in the study. Ten HVs completed the crossed-over studies for the barostat and the IGP measurements (Table 1). Due to adverse events during the test, 2 HVs only completed the cross-over barostat study (cf. infra). Five HVs showed poor tolerance of the barostat procedure, therefore, they could only complete the cross-over IGP measurements.

Table 1: Number of HVs per study arms. This study was single blind randomized cross-over prucalopride (2 mg) vs. placebo.

	Placebo	Prucalopride	Status
IGP + barostat	10 HVs	10 HVs	Completed the entire crossed-over study.
Barostat only	2 HVs	2 HVs	Drop out after the barostat test due to adverse event.
IGP only	5 HVs	5 HVs	Drop out before the barostat test because they did not tolerated the barostat probe.
Total	17 HVs	17 HVs	Included in the study

Gastric barostat study

Conduct of the study

Twelve healthy subjects (58% females, 32±1.7 years old, BMI: 22.8±0.6 Kg/m2) participated in the single-blind randomized controlled cross-over gastric barostat study (placebo vs. prucalopride 2 mg). After the meal, 7 subjects (6 females) were not able to complete the GA measurement and the postprandial gastric distention protocol due to occurrence of adverse events after prucalopride treatment (cf. infra).

Pressures and compliance

The MDP did not differ between both study conditions (10±0.5mmHg vs. 9.8±0.4 mmHg in the placebo and prucalopride arms respectively, p=0.61).

Fasted stepwise isobaric intragastric balloon distentions 2 hours after ingestion of prucalopride (2 mg) or placebo did not show any difference in gastric compliance: (60.7±10.2 mmHg.mL-1 after prucalopride vs. 66.4±10.4 mmHg.mL-1 after placebo (p=0.66)) (Figure 2.A).

Perception scores during the stepwise gastric distentions after prucalopride (2 mg) tended to be higher compared to the placebo (slope prucalopride: 0.8±0.1 mm Hg-1 and slope placebo: 0.6±0.1 mm Hg-1 ; p=0.07) (Figure 2.A).

At the level of the perception threshold the distending pressure (P prucalopride: 4±0.5 mmHg and P placebo: 3±0.5 mmHg, p=0.5) and the corresponding intra-balloon volumes (V prucalopride: 277.8±38.3 mL and V placebo: 308.8±45.4 mL, p=0.4) did not differ significantly between both conditions. Similarly, at the discomfort threshold, there was no significant difference in the distending pressure (P prucalopride: 9±0.8 mmHg and P placebo: 11±0.9 mmHg, p=0.09) and the corresponding intra-balloon volume (V prucalopride: 610.2±55.9 mL and V placebo: 674.5±51.5 mL, p=0.4).

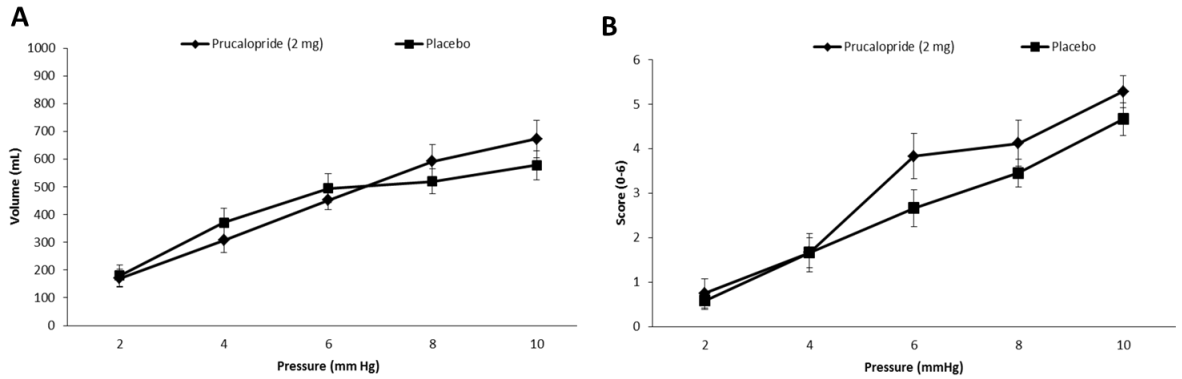


Figure 2. Distention 2 corresponds to the fasting gastric distention 2hs after the ingestion of placebo or prucalopride (2 mg). A. Volume/pressure curve to determine gastric compliance (Slope p=0.66) B. The sensation scores/pressure curve to determine gastric sensitivity to distention (Slope p=0.07) Scores were ranked from 1=perception to 5=discomfort and 6=pain. All data was presented as mean ± SEM.

After the meal, due important nausea and vomiting, 4 HVs (3 females and 1 male) could not complete the postprandial gastric distention test. The postprandial gastric compliance was not significantly different for the evaluable subjects in both treatment groups (Slope prucalopride: 98.6±14.9 ml-1.mmHg and slope placebo: 83.8±11.9 ml-1.mmHg ; p=0.41) (Figure 3.A). In addition, there were no significant differences in postprandial gastric sensitivity to distention in the evaluable patients in both groups (slope of the sensitivity curves and their intercept: Slope prucalopride: 0.8±0.1 mm Hg-1 and placebo: 0.6±0.1 mm Hg-1; p=0.23. Y-intercept prucalopride: -0.1±0.4 and Y-intercept placebo: 0.1±0.7, p=0.78)(Figure 3.B).

At the threshold for first perception, the mean postprandial distending pressure (P prucalopride: 3±0.5 mmHg and P placebo: 2±0.6 mmHg, p=0.5) and the corresponding postprandial intra- balloon volume (V prucalopride: 442±56.1 mL and V placebo: 335.3±63.4 mL, p=0.5) did not differ significantly between both treatment arms. At the discomfort threshold, mean pressures (P prucalopride: 8±1.2 mmHg and P placebo: 11±1.1 mmHg, p=0.1) and corresponding volumes (V prucalopride: 750.2±48.5 mL and V placebo: 685.1±40.6 mL, p=0.5) after treatment with placebo or prucalopride were not altered.

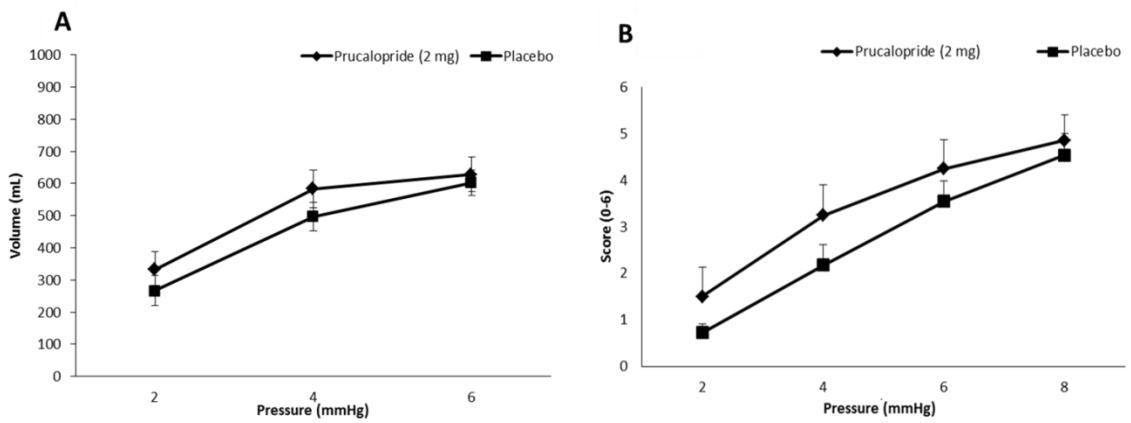


Figure 3. Postprandial gastric distention after the ingestion of 200 ml nutrient drink (300 Kcal). A. Gastric compliance B. Gastric sensitivity to distention score (1=perception; 5=discomfort, 6=pain).

Due to nausea after prucalopride treatment, only 4 volunteers (3 females, 1 male) could not terminate this test. No significant differences in gastric compliance and gastric sensitivity to distention was observed in the evaluable patients (p>0.05). All data was presented as mean ± SEM.

Effect of prucalopride on intragastric volume after a meal

Before the meal, the mean intragastric balloon volume was similar in both groups (V prucalopride: 314.4±49.6 mL and V placebo: 252.1±25.8 mL, p= 0.24).

Ten to fifteen minutes after the meal, 58% of the healthy subjects (6 females and 1 male) had to stop the GA measurement due to excessive feelings of nausea and urge to vomit. During this test, 4 subjects (3 females and 1 male) stopped the study completely due to vomiting. Fifteen minutes after the ingestion of the meal, the intragastric volume was numerically increased in the prucalopride group compared to placebo, yet this difference did not reach statistical significance, possibly because of the sample size (delta prucalopride until 15 min postprandial: 175.7±18.8 mL and delta placebo until 15 min postprandial: 112±36.3 mL respectively, p=0.1) (Figure 4). The maximal postprandial relaxation also tended to be increased in the treatment group compared to placebo (633.8±58.0 mL and 468.6±53.5 mL respectively; p=0.06). However, the time to reach maximal relaxation did not differ between the groups (time prucalopride: 15.8±2.7min and time placebo: 25.8±4.6 min; p=0.1) (Figure 4).

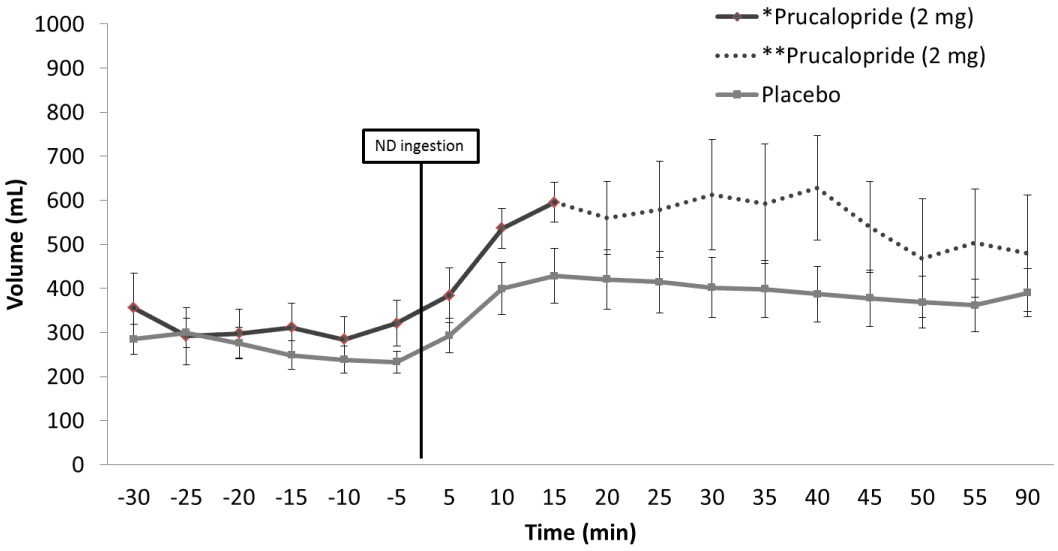


Figure 4: Intragastric volume measurement with the gastric barostat

Prucalopride (2 mg) induce nausea and vomiting in a high proportion of the healthy subjects (58%, 6 females and 1 man). These subjects have to stop the measurement at ±15 min after nutrient drink ingestion. Nutrient drink ingestion (ND, 200 ml) induced gastric accommodation in both treatment groups. However, no significant difference was observed on the prucalopride group 15 min after ingestion of the meal (prucalopride (2 mg) compared to placebo, p=0.1). * Prucalopride (2 mg): 100% of the volunteers could complete the measurement after ingestion of the nutrient drink (15 minutes).**Prucalopride (2 mg): 42% of the volunteers could complete the entire measurement (60 minutes). In all analyses p < 0.05 was considered significant. All data was presented as mean ± SEM.

As 86% percent of the females developed major nausea after prucalopride (2 mg), the results were analyzed according to gender. In females, GA during the first 15 minutes after nutrient drink ingestion was significantly increased after prucalopride (2 mg) compared to placebo (delta prucalopride: 197.6±35.5 mL and delta placebo: 72.4±40 ml, p=0.04) (Figure 5.A). In contrast, males did not show any significant effect on GA after treatment with prucalopride (2 mg) compared to placebo for the first 15 minutes (Delta prucalopride (2 mg): 167.4±63.4 ml and delta placebo: 142.8±19.9 ml, p=0.73) (Figure 5.B).

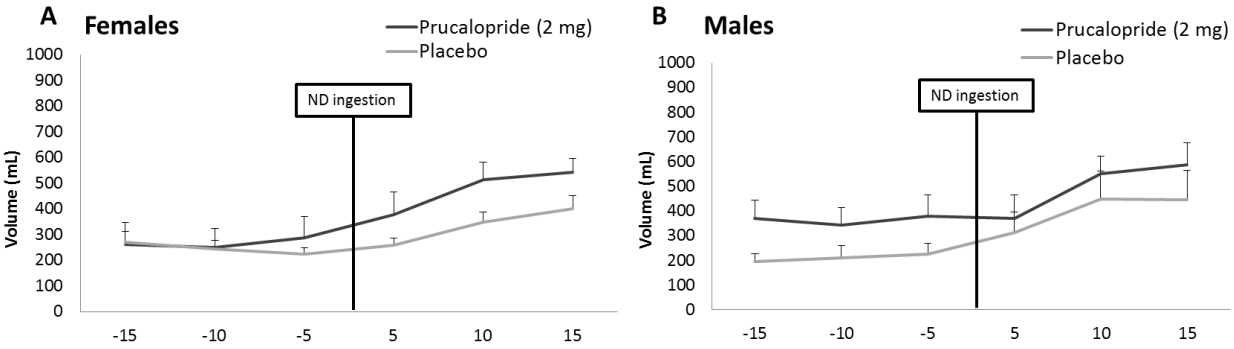


Figure 5: Intragastric volume measurement with the gastric barostat males vs. females. A. In females prucalopride significantly increased the gastric accommodation after ingestion of nutrient drink (p=0.04) compared to placebo. Prucalopride (2 mg) induce nausea and vomiting in a high proportion of females (86%). These subjects have to stop the measurement at ±15 min after nutrient drink ingestion. B. This effect was not observed in males (p>0.05). In all analyses p < 0.05 was considered significant. All data was presented as mean ± SEM.

Gastric motility index

No difference was observed in the gastric motility index before the meal in both groups (normalized AUC for the prucalopride group 45.2±8.4 mL.min-1 vs. 42.1±4.6 mL.min-1 for the placebo group; p=0.74) (Figure 6.A). Fifteen minutes after the meal, the motility index increased in the prucalopride group compared to placebo, but this did not reach statistical significance (normalized AUC prucalopride: 67.5±5.8 mL.min-1 vs. placebo: 49.4±6.2, p=0.08).

Effect on symptoms during the gastric barostat test

During the barostat study after prucalopride all measured epigastric symptoms were numerically increased compared to placebo (Figure 7.A.). However, in the fasted state these results were not significantly different compared to placebo. From the moment of meal ingestion until 15 min after, hunger ratings were clearly decreased (p=0.02) and epigastric symptoms were increased in the prucalopride group (upper abdominal bloating (p=0.04), postprandial fullness (p=0.09), nausea (p=0.0002) and cramps (p=0.06)). Major nausea was induced in 7 healthy subjects (6 females) who had to stop the GA measurement prematurely (on average 19±2.9 min after the meal). Four of these subjects (3 females) also vomited during the test.

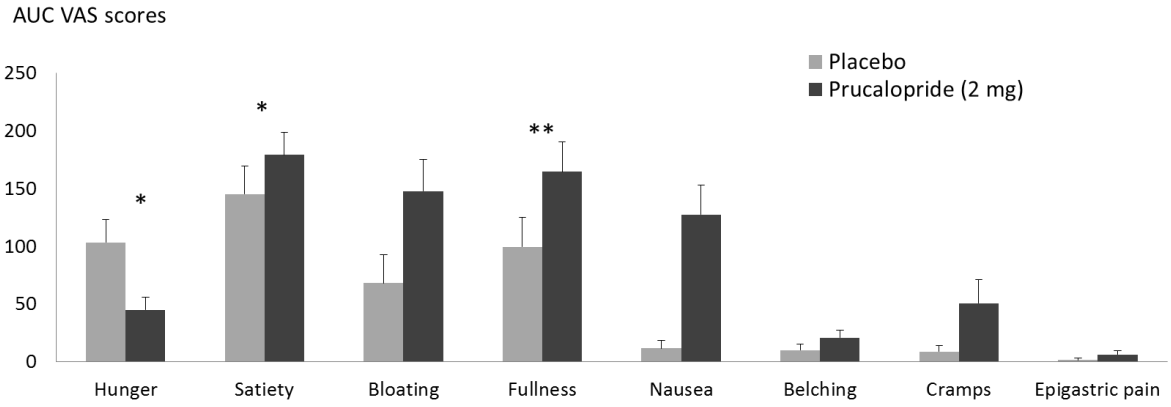


Figure 7.A. VAS scores of epigastric symptoms. The postprandial AUC of the VAS score for hunger was clearly decreased (p=0.02) and upper abdominal bloating (p=0.04), postprandial fullness (p=0.09), nausea (p=0.0002) and cramps (p=0.06) were increased.*p<0.05; **p<0.001.

In the prucalopride treatment group, a correlation was found between the increasing nausea VAS scores and the increasing intra-balloon volumes from the moment the drink was ingested until 15 minutes postprandial compared to placebo (Prucalopride spearman r=0.37, p=0.03 and placebo spearman r: 0.25, p=0.14) (Figure 7b).

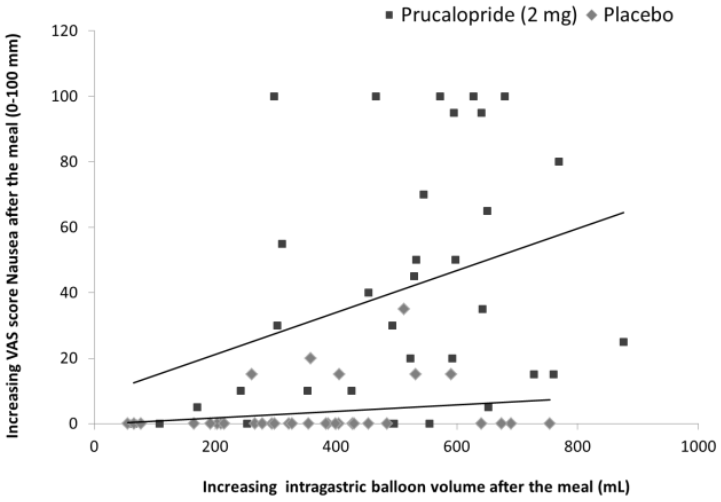


Figure 7. B. Correlation of increasing nausea VAS scores (0-100 mm) and increasing intragastric balloon volume (ml) after ingesting of the liquid meal (200ml, 300 Kcal). Postprandial nausea VAS scores increased significantly with the increasing intragastric volume during prucalopride treatment, but not during placebo (Prucalopride spearman r=0.37, p=0.03 and placebo r: 0.25, p=0.14).

Intragastric pressure measurement

Conduct of the study

Fifteen healthy subjects (67% females, 26.7±1.7 years old, BMI: 22.2±0.6 Kg/m^2) participated in this single blind randomized (placebo vs. prucalopride (2 mg)) HRM intragastric pressure measurement study. Ten of these volunteers also participated in the gastric barostat study.

Effect of prucalopride on proximal stomach IGP during intragastric meal infusion

Two hours after treatment with prucalopride (2 mg) or placebo, the ingestion of the meal induced a drop in proximal stomach IGP from baseline, followed by a gradual recovery (Figure 8). The area above the curve (AAC) was found to be comparable in both treatment groups (AAC prucalopride: -16.24±5.10 mmHg.min and AAC placebo: -18.25±3.71 mmHg.min, p=0.67). Moreover, the nadir did not differ between the groups (prucalopride: -4.79±0.93 mmHg vs. placebo: -5.49±0.76 mmHg, p=0.43), nor did the time to reach nadir (prucalopride: 5.0±0.86 min and placebo: 7.67±1.21 min; p=0.09).

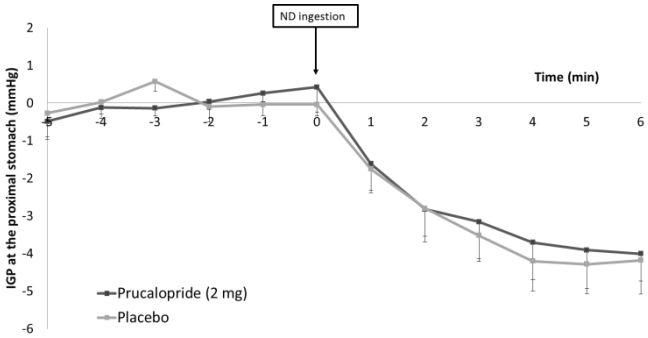


Figure 8. IGP curves in the proximal stomach were not different after treatment compared to placebo. The nadir and time to nadir did not differ between the groups (p>0,05).

Effect of prucalopride on distal stomach intragastric pressure before and during intragastric meal infusion

When observing the antral intragastric pressure changes over time before the meal, a significant increase of IGP was observed between the 60 minutes and 95 minutes after the ingestion of prucalopride (2 mg) compared to placebo (p<0.05) (Figure 9).

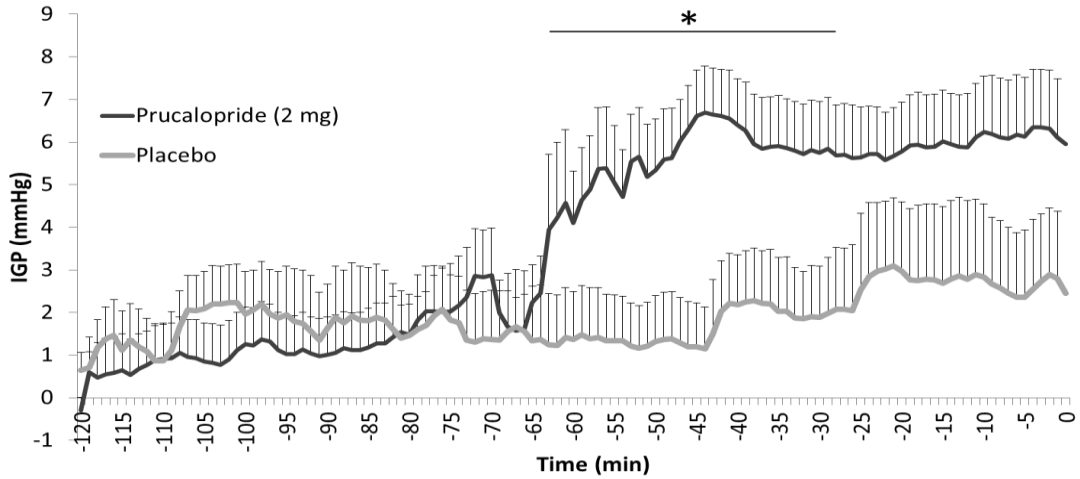


Figure 9. Antral IGP was increased significantly between the 60 minutes and 95 minutes after the ingestion of prucalopride (2 mg) compared to placebo (p<0.05).

During the first hour after prucalopride (2 mg), the area under the IGP curve during prucalopride treatment was not significantly increased compared to placebo (AUC prucalopride: 78.4±43.4 mmHg.min and AUC placebo: 95.1±55.3 mmHg.min; p=0.7). During the second hour before the meal,

the AUC was increased after prucalopride treatment compared to placebo (AUC prucalopride: 345.6±57.2 mmHg.min and AUC placebo: 126.7±80 mmHg.min; p=0.05).

During this period, the antral contractile pattern of the migrating motor complex (MMC) was observed. The MMC includes phase II contractions that are described as stationary irregular contractions and phase III contractions that are characterized by short clustered contraction (296).

Phase II contractions were observed during prucalopride (n=14) and placebo (n=13) treatment. After ingestion of placebo, the contractions started at 54.0±10 minutes. During the studies (120 minutes of measurement), the average length of this contraction period was of 39.5±5.3 minutes. In average the amplitude of these contractions was of 335.9±25.1 mmHg (Figure 10.A). During the treatment of prucalopride, phase II contractions started at an average of 42.9±4.9 minutes after ingestion of prucalopride and had an average length of 42.7±7.7 minutes (Figure 10.B). The average amplitude of these contractions was of 345.9±24.5 mmHg. However, these differences were not significant.

The phase III contractions were observed in 7 subjects in the placebo group and they started 50±14.6 minutes after the ingestion of placebo. The average amplitude of these contractions was 342±44 mmHg. During placebo the duration of these contractions was of 5.3±1.1 minutes (Figure 10.C).The same type of contractions was observed in 9 subjects at an average of 73.7±3.3 minutes after ingestion of prucalopride. The average amplitude of these contractions was 340.5±27.3 mmHg (Figure 10.D). In the prucalopride group the period of the contractions was similar to placebo; 7.2±2.6 minutes and 5.3±1.1 minutes (p>0.05).

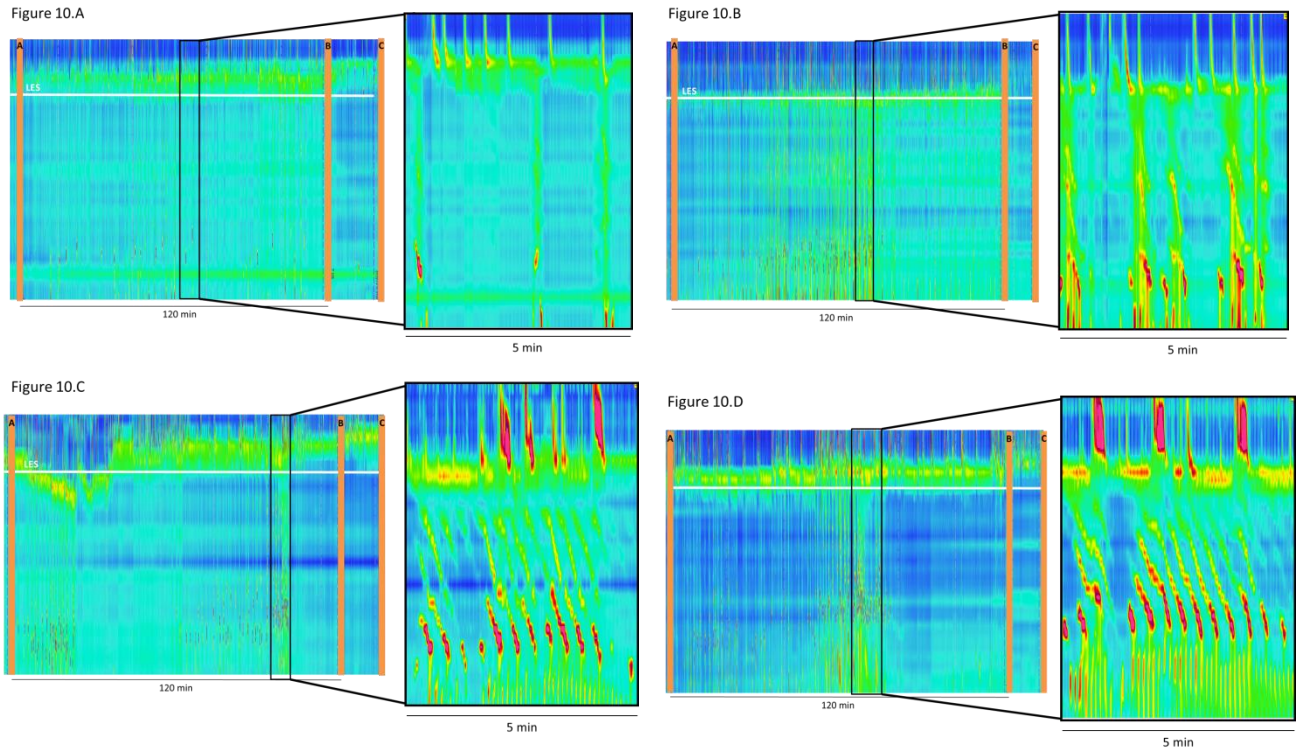


Figure 10. A. Example Phase II during placebo treatment in HV6 (Range= max: 200 mmHg, min -15 mmHg). B. Example Phase II during prucalopride treatment in HV6 (Range= max: 200 mmHg, min -15 mmHg). Phase II contractions can be described as stationary irregular antral contractions in 13 HVs. The frequency and amplitude of these contractions was slightly increased after prucalopride

treatment compared to placebo, however, not significant. C. Example Phase III during placebo treatment in HV3 (Range= max: 200 mmHg, min -15 mmHg). D. Example Phase III during prucalopride (2 mg) treatment in HV3 (Range= max: 200 mmHg, min -15 mmHg). Phase III contractions that are characterized by short clustered antral contraction in 7 HVs after placebo and in 9 HVs after prucalopride. The amplitude of antral contractions was not significantly different compared to placebo.

The ingestion of the meal also induced a small drop in distal IGP from baseline, followed by a gradual recovery (n=14) (Figure 11). Compared to the proximal IGP drop, the distal stomach IGP drop was significantly decreased in the prucalopride treatment group. In the placebo group the proximal IGP AAC was -18.3±3.7 mmHg.min and the distal IGP AAC was -15.8±3.5 mmHg.min (p=0.58); after prucalopride, the proximal IGP AUC -16.2±5.1 mmHg.min and the distal IGP AUC was -8.1±6.3 mmHg.min (p=0.02). However, the comparison of drop in distal stomach IGP between placebo and prucalopride did not reach statistical significance (p=0.20). Moreover, the nadir and the time to reach nadir also did not differ significantly between the treatment groups (nadir prucalopride: -4.02±0.92 min and placebo: -5.0±0.86 min; p=0.37; time to reach nadir prucalopride: 7.28±1.00 min and placebo: 7.07±0.92 min; p=0.88).

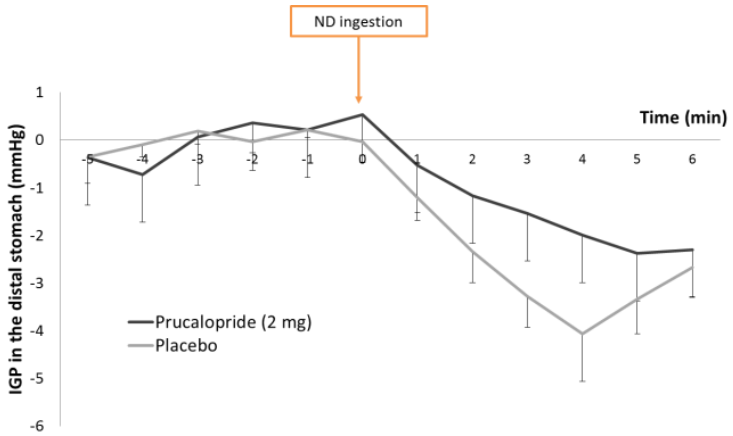


Figure 11. IGP placebo vs. prucalopride in the distal stomach. The ingestion of the meal also induced a small drop in distal IGP from baseline, followed by a gradual recovery. Nadir and time to nadir did not differ between groups.

Effect of prucalopride on satiation during intragastric infusion of ND

There was no difference in nutrient tolerance and time to reach maximal satiation after treatment with prucalopride or placebo. The mean maximal tolerated volume was 568±55.82 mL after prucalopride treatment and 676±62.91 mL after placebo (p=0.13).

Effect on symptoms during the IGP measurement

During the manometry study, the VAS scores showed only an increase on epigastric cramps after treatment with prucalopride (2 mg) (Figure 12). The increase on antral IGP was associated with the increase on cramps severity scores (r=0.78, p<0.0001). This association was not found for antral IGP and VAS cramps scores in the placebo treatment.

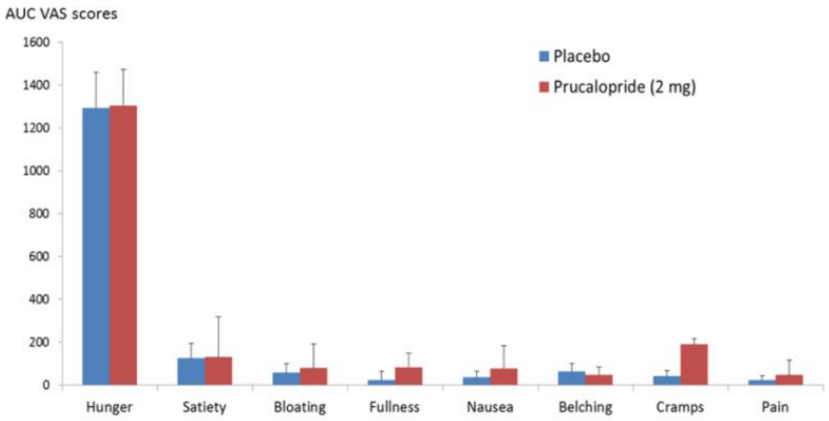


Figure 12. AUC of VAS symptoms scores during IGP measurements. The up come of epigastric cramps were significantly increased after treatment with prucalopride (2 mg)(p=0.05). Epigastric pain tended to increase as well in the treatment group compared to placebo (p=0.08). Values were compared with the student's T-test. The results were presented as mean ± SEM.

5.2.4. Discussion

In PDS patients, disorders of gastric sensorimotor function, such as impaired accommodation, delayed gastric emptying and hypersensitivity to gastric distention, have been implicated in symptom generation (16, 22, 58, 62, 78, 189-191). Based on this assumption, prokinetics are recommended as initial therapeutic approach for PDS patients (96, 222)

Additionally, it has been previously reported that variations in serotonin levels might play a role in GI disorders (297, 298). Increased and decreased levels of serotonin in the gastrointestinal tract might have been previously described in inflammatory disorders such as coeliac disease (increased levels) and ulcerative colitis (decreased levels) (297, 298). In functional GI disorders such as IBS, decreased expression of SERT transporters has been reported, but not all studies confirmed this (24, 297, 298).

5-HT_4 receptor agonists such as cisapride and tegaserod have been evaluated for the treatment of upper gastrointestinal motility disorders, including functional dyspepsia and gastroparesis (24, 26, 30, 118, 119, 163, 222, 299). They stimulate motility by enhancing acetylcholine release from myenteric neurons in the gut wall, and have been considered of potential use in (a subsets of) FD patients. However, due to cardiovascular side effects these drugs have been withdrawn from the market (115).

Prucalopride is a novel and safe highly selective $5HT_4$ receptor agonist which is approved for the treatment of chronic constipation (286, 288, 289). Previous studies have demonstrated the potential for prucalopride to enhance gastric emptying and small bowel transit in health and disease (283, 284, 288-290, 300). In the present study, we evaluated the effect of prucalopride on gastric motility and sensitivity by means of the gastric barostat and intragastric pressure studies.

The gastric barostat allows isobaric or isovolumetric distention of the stomach and, under isobaric conditions, it measures the air volume in the balloon maintained at a constant pressure. This technique has been used for many years as the gold standard to measure gastric sensitivity and gastric accommodation. Nevertheless, the procedure is invasive, uncomfortable and difficult to tolerate (155, 159, 257). Studies have also previously shown that the presence of the barostat bag may alter the intragastric distribution of a meal and it might exaggerate the relaxation of the proximal stomach due to the direct distending effect of the balloon on the stomach wall (158, 159). Actually, in our study we observed that before the meal, the mean of the intragastric balloon volume showed a tendency to

increase in the treatment group compared to placebo, although this did not reach significance. The maximal relaxation tended also to be increased in the treatment group compared to placebo. HVs reported higher ratings for symptoms after treatment with prucalopride, but only nausea levels were significantly increased compared to placebo. During the barostat in the prucalopride arm, after the meal, a large group of predominantly female volunteers experienced excessive feelings of nausea, with vomiting in some of them, necessitating interruption of the measurement. In this group, a correlation was found between the increase of the nausea VAS scores and the increase of the intra-balloon volumes in the first 15 minutes postprandial compared to placebo.

The literature shows that the most prevalent side effects of prucalopride are headache, nausea and diarrhea and these increase dose dependently (287, 289). However, no significant increase of side effects is observed above 4 mg prucalopride, suggesting the attenuation of the signal on the receptors (283). Nausea is associated with the desire to vomit and a number of physiological changes such as proximal stomach relaxation (301). The latter effect could explain the large stomach relaxation observed during our measurements with the gastric barostat. There is some evidence that 5-HT_4 receptor activation may lead to emesis (301-303). In dogs and ferrets, emesis caused by copper sulphate is mediated by 5-HT_4 receptor activation (302). Copper-induced emesis depends on vagal nerve function and 5-HT_4 receptor activation can also depolarize vagus nerve preparations, at least in rodents (302).

Furthermore, the barostat results showed that prucalopride did not affect proximal stomach compliance. Moreover, fasting sensitivity to isobaric balloon distention was slightly enhanced by prucalopride ($p= 0.07$). These results agree with a study with another serotonin agonist, cisapride, in HVs with a 5 day pre-treatment also showed an increase in gastric perceptions but no difference in gastric compliance (26).

Taken together, these observations suggest that the gastric volume measurements after the meal during treatment with prucalopride may reflect nausea-related events, induced by prucalopride in the presence of a distending barostat bag in the stomach, rather than a true effect of prucalopride on the proximal stomach of the subjects. This interpretation is supported by our observations in the IGP studies.

The measurement of intragastric pressure by means of the high resolution manometry has previously been implemented as a minimally invasive alternative to the gastric barostat to assess gastric accommodation (160). This technique comprises a catheter composed of 36 closely spaced pressure sensors to measure intraluminal pressure changes from the LES to the duodenum. During the intragastric infusion of a liquid nutrient meal, the IGP decreases rapidly and gradually recovers, reflecting gastric relaxation upon nutrient infusion (160, 166, 258, 259). This technique is easy to tolerate and easy to perform, it provides information of IGP before, during and after the meal. Moreover, it does not only generate information about pressures in the proximal stomach, but also the pressures at the LES and at the distal stomach, and, unlike the barostat, there is no reason to assume that it might interfere with the normal distribution of the meal in the stomach.

In this study, during intragastric pressure measurements no differences were found in gastric IGP drop and nutrient tolerance after treatment compared to placebo. VAS scores of nausea were not enhanced by the treatment compared to placebo, but a significant increase of epigastric cramps was observed. Intragastric pressure measurement of antral contractions and the motility index after prucalopride

treatment showed to be significant increased compared to placebo. In addition, this was significantly correlated to increased cramp scores after 1h of prucalopride treatment.

This effect on gastric contractility was previously described for other serotonin agonists (304-306). Cisapride was shown to promote contractile activities throughout the gastrointestinal tract, and it enhances gastric emptying by increasing both the amplitude and the number of antral waves that propagate to the duodenum (290, 307-309). Cisapride was also shown to enhance the meal-related gastric relaxation of the proximal stomach (26). As a potential mechanism, enhanced antral contractility of the gastric antrum induced by cisapride could induce a redistribution of the meal towards the proximal stomach.

After reviewing the results of this study, the question remains as to whether prucalopride might be a good alternative treatment for FD. At this point, it can only be speculated that prucalopride might lead to a beneficial effect in FD patients with delayed gastric emptying, as it seems that this drug increases antral activity. Nevertheless, exacerbation of epigastric symptoms and increased gastric sensitivity during the first days of treatment could potentially occur based on the results of our studies.

In conclusion in this study we showed that prucalopride may increase sensitivity to gastric distention in healthy subjects. However, it does not enhance gastric accommodation after a standard meal or increase nutrient tolerance. Prucalopride enhances gastric distention-induced nausea during the gastric barostat study in healthy subjects, suggesting that in this case the presence of the gastric barostat might influence the measurements of gastric accommodation.

5.3 The effect of mirtazapine on gastric accommodation and gastric sensitivity in healthy volunteers.

5.3.1. Introduction

In functional dyspepsia (FD), the postprandial distress syndrome (PDS) as defined by the Rome consensus is the largest subgroup, comprising an estimate 30-60% of all FD patients (42, 43, 53, 54, 169). PDS is characterized by meal-related symptoms such as early satiation and postprandial fullness which, over time, may lead to important weight loss. Indeed, it has previously been shown that a strong association exists between weight loss and symptoms including early satiation, both in the general population and in FD patient samples (22, 230). It is has also been shown in PDS patients that the presence of psychological distress such as anxiety and somatization, is associated with higher (meal-related) symptom severity and weight loss (48, 60). To date, prokinetic agents are considered the preferred treatment option for PDS patients, through their putative ability to improve disturbances of gastric motor function such as impaired gastric accommodation and delayed gastric emptying (16, 21, 22, 58, 61, 190). However, the availability of prokinetics is limited and for most of them there is lack of studies convincingly demonstrating their ability to provide substantial symptom relief. Psychotropic agents, such as anxiolytics and antidepressants, are also used for the management of FD symptoms, partly because of their ability to improve psycho-social co-morbidity, their favorable effect on sleep, and their potential to act as central analgesic agent (15). Furthermore, a number of studies have shown that they are also able to modulate gastrointestinal sensorimotor function (27, 28, 96, 138, 310).

Mirtazapine is an antidepressant of the newest generation with central noradrenergic and serotonergic activity. It presynaptically blocks inhibitory α2-adrenergic autoreceptors and heteroreceptors, leading to enhanced norepinephrine and 5-HT_{1A} serotonergic neurotransmission (311, 312). Postsynaptically, mirtazapine blocks 5-HT_{2C} and 5-HT_3 receptors and it has a high affinity for histamine H_1 receptors (311, 312). In the literature, it has been reported that mirtazapine stimulates appetite and weight gain and, it reduces nausea in different patient populations, including anorexia nervosa and cancer (140, 141, 311, 312). This induced weight gain might be associated to the activation of H_1 receptors and to the increase of fat mass by the increase of leptin levels (311-313). Mirtazapine seems also to enhance gastrointestinal transit. It has been reported that mirtazapine improved symptoms of gastroparesis unresponsive to conventional prokinetics (142-144, 314, 315). In a recent controlled study in FD patients with weight loss, mirtazapine was shown to improves symptoms and increase nutrient tolerance (139). However, it remains to be established whether mirtazapine does this through potential effects on gastro-sensorimotor function. Hence, the objective of this study was to evaluate the effect of mirtazapine on gastric sensorimotor function in healthy volunteers.

5.3.2. Materials and Methods

Subjects and study design

Healthy volunteers (HVs), recruited by public advertisement, were invited to participate in a single blind randomized parallel-group study with mirtazapine (15 mg, Remergon®, Belgium) and placebo.

The study comprised two gastric sensorimotor function measurements: a gastric barostat study and an intragastric pressure (IGP) measurement study with high resolution manometry (HRM). Measurements

were done before and at the end of the treatment period. Treatment consisted of a 3-week dosing of mirtazapine (15 mg) or placebo every night before sleeping for 3 weeks (Figure 1). During these 3 weeks of treatment, gastrointestinal symptoms or side effects were tracked using daily diaries.
HVs had to be devoid of GI symptoms and of the use of medications known to influence gastrointestinal sensorimotor function. The study was approved by the Ethics Committee of the University Hospitals, Leuven, Belgium and informed consent was obtained from all subjects before entering the study.

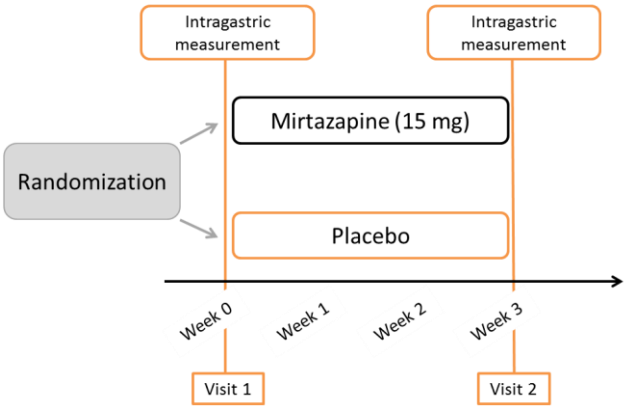


Figure 1: Overview single-blind parallel placebo vs. mirtazapine (15 mg) study. Intragastric measurements (gastric barostat and HRM) were done at baseline (visit 1) and after 3 weeks of treatment at visit 2.

Gastric barostat study

Following an overnight fast of at least 12 h, a double lumen polyvinyl tube (Salem sump tube 14 Ch., Sherwood Medical, Petit Rechain, Belgium) with an adherent plastic bag (1200 mL capacity) which was finely folded, was introduced through the mouth and secured to the subject's chin with adhesive tape. The HVs were then asked to take placed in a specifically designed bed in a sitting position with the knees slightly bent. The polyvinyl tube was connected to a computer-driven programmable volume-displacement barostat device (G&J Electronics Inc., Toronto, ON, Canada). To unfold the intragastric bag, it was inflated with a fixed volume of 300 mL of air for 2 min and again deflated completely.
After a 10 min equilibration period, the minimal distending pressure (MDP) was determined by increasing the intrabag pressure by 1 mmHg every minute until the intrabag volume of 30 mL or more was stable for 2 minutes.
For the evaluation of the gastric sensitivity and compliance, stepwise isobaric distentions (increments of 2 mmHg every 2 minutes starting from the MDP) were initiated. Distentions were performed before treatment, at fasted state and after the meal challenge (postprandial state). At every distending step, the subjects were instructed to rate the intensity of upper abdominal sensation (0: no sensation – 5: discomfort, 6: epigastric pain) induced by every stimulus. The procedure concluded when subjects reported maximal discomfort or pain (score 5-6) or when the intrabag volume reached 1000 mL.
For the meal challenge the pressure level was set at MDP + 2 mmHg. After a 30 min baseline period, HVs ingested 200 ml of a nutrient liquid meal (Fortimel Energy®, Nutricia, 150 kcal per 100 ml with 5.9 g proteins, 18.4 g carbohydrates and 5.8 g lipids, Netherlands). Measurement continued for 60 minutes postprandial. Subsequently, the distention series was repeated. During the entire accommodation study, volunteers were asked to fill out visual analogue scales (VAS) for hunger,

satiation and 6 epigastric symptoms (fullness, nausea, belching of air, cramps in the abdomen, bloating and pain) at 5-minute intervals.

Intragastric pressure measurement study

A high resolution manometry probe (HRM, 36 pressure measurement points, ManoScan 360, Sierra Scientific instruments, Los Angeles (USA)) was passed through the nose into the distal stomach of the HVs. To infuse the nutrient drink directly into the stomach, a second nasogastric feeding tube (Enteral™, Maxter-catheters, Marseille, France) was positioned through the nose into the proximal stomach. The position of the catheters was verified briefly by fluoroscopy. The catheters were fixed to the subjects' nose and the subjects were placed in a bed in a comfortable sitting position with the knees slightly bend. After a stabilization period of 10 minutes, IGP baseline was measured for 30 min. Hereafter, a nutrient drink (Fortimel Energy®, Nutricia, 150 kcal per 100 ml with 5.9 g proteins, 18.4 g carbohydrates and 5.8 g lipids, Netherlands) was infused directly into the stomach of the HVs at a constant speed of 60 ml/min. During the study, volunteers were asked to fill out visual analogue scales (VAS) for hunger, satiation and 6 epigastric symptoms (fullness, nausea, belching of air, cramps in the abdomen, bloating and pain) at 5-minute intervals. In addition, during nutrient drink infusion they also scored the level of satiation at 1-minute intervals by using a graphic rating scale that combines verbal descriptors on a scale graded from 0–5 (5, maximum satiation).

Intragastric infusion was stopped as soon as the HVs reached the maximum score of 5 on their satiation scale or when they score maximally on one of the epigastric symptoms. Five minutes hereafter the catheters were disconnected and removed and the volunteers could leave the hospital.

Data Analysis

Gastric barostat study

During the gastric sensitivity studies, for each 2-minute distending period, the mean intragastric volume was calculated. The perception threshold was defined as the first level of pressure and the corresponding volume that evoked a perception score of 1 or more. Discomfort threshold was defined as the first level of pressure and the corresponding volume that provoked a sensation score of five or more. The gastric compliance of the subjects was calculated as the slope of the volume/pressure curve. The gastric sensitivity to distention of the subjects was calculated as the slope of the sensitivity scores/pressure curve.

Gastric tone before and after administration of the meal was measured by the calculation of the mean balloon volume for consecutive 5-min intervals. Fasting volume (baseline) was measured as the mean volume of the 30 min period before ingestion of nutrient drink and the mean postprandial volume was defined as the mean volume one hour after ingestion of the nutrient drink (200 ml). Measurements were done by using commercially available software (Protocol plus™ and Protocol plus™ data scanner). The meal-induced accommodation response was determined as the difference (delta) between the mean volume before (30 min) and after meal intake (60 min).

The visual analogue scales (0-100 mm) for epigastric symptoms (fullness, nausea, belching of air, cramps in the abdomen, bloating and pain) at 5-minute intervals for the mirtazapine group and placebo group were compared by the student t-test of pre- and postprandial area under the curve (AAC) of the symptoms scores over time (10 minutes before and 10 min after the meal).

In all analyses ***P***-value of <0.05 was considered statistically significant. Calculations were compared for treatment versus placebo by paired ***t***-test and correlations were done with Spearman's rank correlation coefficient. All data are presented as mean ± SEM.

Intragastric pressure measurement study

The IGP original data was imported from the recorder software ManoAcquisition® to Excel. The data was calculated as previously described by Jansen *et al*. The proximal IGP was measured as the average pressure of the first five pressure channels that were clearly positioned below the lower esophageal sphincter (LES) or the pressure area influenced by the LES. The distal IGP was measured as the as the average pressure of five pressure channels that were clearly positioned in the distal part of the stomach, characterized by antral contractions during the fasted state.

To avoid influence from movements such as swallowing, movement, etc., a moving median was calculated from the original data (median value over 1 minute of the original data). Per channel, a baseline value was calculated from the moving median data corresponding to the minimum pressure in the last 5 minutes of the stabilization period before nutrient drink infusion. The paired t-test was used to compare the mean AAC between the IGP curves of the mirtazapine and placebo groups. The nadir IGP was defined as the minimal IGP or the lowest relaxation point during nutrient drink infusion. Mean AAC satiation scores curves and the mean volume and time to reach maximal satiation were compared with the paired t-test. The slope of the satiety score curve was calculated by linear interpolation and compared between groups with the paired t-test.

The visual analogue scales for upper abdominal symptoms (fullness, nausea, belching of air, cramps in the abdomen, bloating and pain) at 5-minute intervals for the mirtazapine group and placebo group were compared with the paired t-test. In all analyses $p < 0.05$ was considered significant. All data are presented as mean ± SEM.

5.2.3. Results

Study subjects demographics

The demographics of the 31 included HV are shown in table 1. There were no significant differences between both treatment groups. All HVs tolerated and participated in IGP measurements. Two of these volunteers did not tolerate the gastric barostat and were not able to complete the entire measurement (Table 1).

Three weeks after treatment with mirtazapine, the weight of the volunteers increased significantly from 67.8±3.7 Kg to 69.1±3.7 Kg (p=0.01). This increase was not observed in the placebo group (baseline: 70.8±3.1 Kg, 3 weeks after treatment: 71.2±3.2 Kg; p=0.35).

Table 1: Number of HVs with demographics per study arms. This was a single blind randomized parallel mirtazapine vs. placebo study.

	Placebo	Mirtazapine	Status
Total (n=31)	15 HVs	16 HVs	1 HV withdrawn due to severe adverse event during 1st week of mirtazapine treatment phase.
Age	24.9±1.0	23.9±1.3	
% Females	60%	60%	
BMI (Kg/m^2)	23.4±0.8	22.4±0.7	
IGP measurement (n=30)	15 HVs	15 HVs	All HVs finalized the study.
Age	24.9±0.96	23.9±0.86	
% Females	60%	60%	
BMI (Kg/m^2)	23.2±0.93	22.4±0.54	
Barostat (n=28)	14 HVs	14 HVs	2 HVs drop out of the barostat study because they not tolerated the barostat probe.
Age	25.14±1.06	24.07±1.39	
% Females	53%	53%	
BMI (Kg/m^2)	23.72±0.82	22.49±0.73	

Adverse events

The principal adverse events were headache (NS), fatigue (p<0.001) and dizziness (p=0.02). Both, in the placebo and mirtazapine treatment groups 13% of the volunteers experienced symptoms of a mild gastroenteritis that included nausea and vomiting on the first week of treatment. The severity of these adverse events was minimal (Table 2). One HV was withdrawn from the study, due to an urticarial rash, which disappeared 3 days after the treatment with mirtazapine was stopped.

Table 2: Overview adverse events in the placebo and mirtazapine groups.

AE	Placebo	Mirtazapine (15 mg)	P-value
Headache	20%	27%	NS
Gastroenteritis	13%	13%	NS
Fatigue	13%	53%	<0,001
Dizziness	0%	7%	0,02
Skin rash	0%	7%	0,02

Gastric barostat study

No differences were observed in MDP between placebo and mirtazapine groups at baseline (p=0.78). The MDP was similar before and after 3 weeks of placebo (9.3±0.4 mmHg vs. 9.1±0.3 mmHg, p=0.73) as well as before and after 3 weeks of treatment with mirtazapine (9.2±0.5 mmHg vs. 9.5±0.4 mmHg, p=0.48).

The effect of mirtazapine on gastric compliance

Baseline stepwise isobaric intragastric balloon distention measurements in the placebo and mirtazapine group were comparable: the slope of the pressure-volume distention curves at baseline in the placebo group was 98.8±11.9 mL.mmHg $^{-1}$ and at baseline before mirtazapine treatment was 85.9±9.2 mL.mmHg $^{-1}$ (p=0.47) (Figure 2.a.).

In the placebo group fasting stepwise isobaric distentions did not show any difference in gastric compliance (3 weeks after treatment 103.1±17.1 mL.mmHg $^{-1}$; p=0.82). After 3 weeks of mirtazapine treatment, also no difference in gastric compliance was observed (73.5±9.1 mL.mmHg $^{-1}$; p=0.37) (Figure 2.b.). Postprandial gastric compliance was also not affected after placebo and mirtazapine treatment (data not shown).

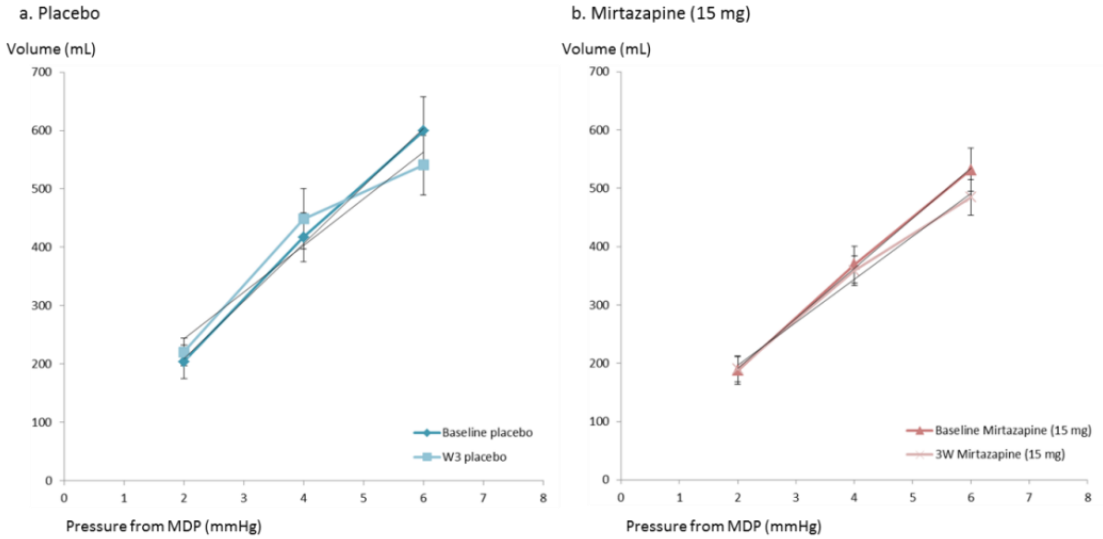


Figure 2: Preprandial gastric compliance was not affected after (a) placebo and (b) mirtazapine treatment. The slope after treatment mirtazapine (73.5±9.1 mL.mmHg-1) compared to baseline (85.9±9.2 ml.mmHg-1) was not significantly different (p=0.37).

The effect of mirtazapine on Gastric sensitivity to distention

At baseline sensitivity to stepwise isobaric intragastric balloon distention was comparable between placebo and mirtazapine subgroup (see table 3).

After 3 weeks treatment with placebo, no differences were observed compared to baseline (Figure 3.a.). After 3 weeks treatment with mirtazapine, the pressure at perception threshold was decreased compared to baseline (2.86±0.27 at baseline vs. 2.29±0.19 mmHg after 3 weeks of mirtazapine; p=0.04). No difference was observed for the discomfort pressure (9.14±0.58 mmHg; p=0.23). After 3 weeks of mirtazapine treatment, the slope of the perception score-pressure curve and the Y-intercept were not significantly different from baseline (table, Figure 3.b.). After the meal, the gastric sensitivity to distention was not affected after placebo and mirtazapine treatment (data not shown).

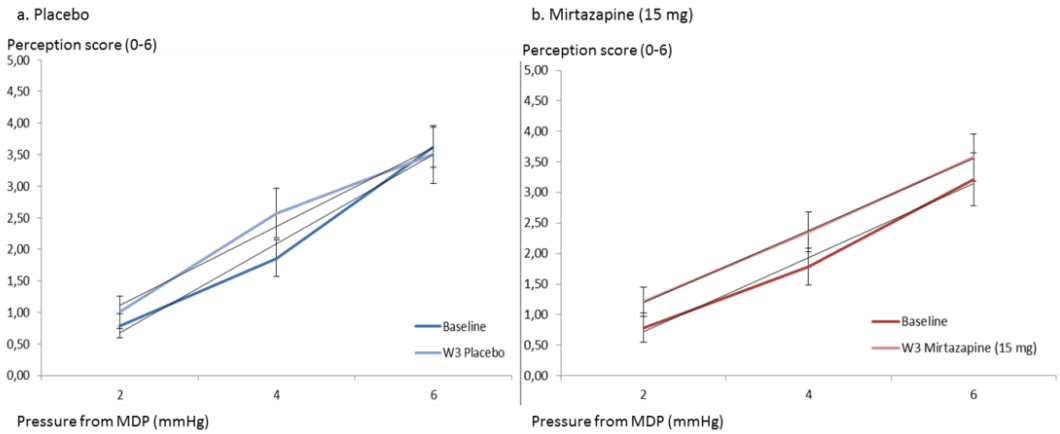


Figure 3: Preprandial gastric sensitivity to distention was not affected after (a) placebo and (b) mirtazapine treatment. The slopes were similar before and after 3 weeks of treatment with mirtazapine (0.59±0.08 mmHg; p=0.86). Moreover, the pressure at perception score was decreased compared to baseline (2.29±0.19 mmHg; p=0.04) and no differences was observed for the discomfort pressure (9.14±0.58 mmHg; p=0.23).

Table 3: Overview values of the placebo and mirtazapine groups during the gastric sensitivity distention steps at baseline and after 3 weeks of treatment.

		Placebo	Mirtazapine
Baseline	Sensitivity to distention (slope, mmHg)	0.70±0.07	0.61±0.10
	Pressure at perception (mmHg)	3.00±0.4	2.86±0.27
	Pressures at discomfort (mmHg)	8.14±0.61	10.0±0.86
	Y-intercept	-0.71±0.28	-0.50±0.32
After 3 weeks of treatment	Sensitivity to distention (slope, mmHg)	0.77±0.11	0.59±0.08
	Pressure at perception (mmHg)	2.71±0.26	2.29±0.19
	Pressures at discomfort (mmHg)	7.85±0.68	9.14±0.58
	Y-intercept	-0.52±0.29	0.02±0.28

The effect of mirtazapine on intragastric volume after a meal

At baseline, the meal-induced increase in intragastric balloon volume (accommodation) was similar in both treatment groups (placebo: 271.49±42.67 mL and mirtazapine: 206.08±50.5 mL, p= 0.24).

No differences were observed after 3 weeks of treatment with placebo (297.17±40.65 mL; p=0.69). The maximal relaxation was also not different compared to baseline (baseline 704.46±55.43 mL and after placebo 677.66±56.93 mL; p=0.84) (Figure 4.a.). After 3 weeks of treatment with mirtazapine, the intragastric barostat balloon volume was not significantly altered (216.23±29.25 mL; p=0.85). Maximal relaxation was similar at baseline 591.45±72.84 mL and after 3 weeks mirtazapine 656.66±44.82 mL (p=0.36)(Figure 4.b.).

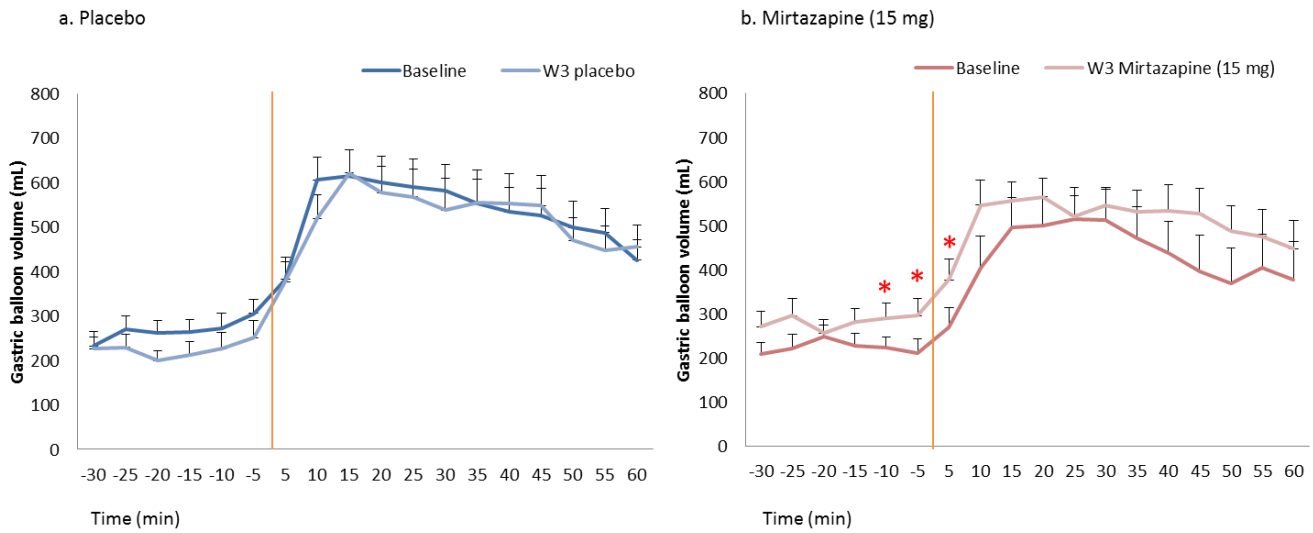


Figure 4: Gastric accommodation during gastric barostat measurement. Subjects drank 200 ml of a nutrient drink (300 Kcal) at time point 0 (orange line). After 3 weeks of treatment, intragastric volume seemed increased. However, delta (Δ) of the mean pre- and postprandial intragastric balloon volume were similar (Δ after 3 weeks mirtazapine: 216.23±29.25 mL; p=0.85). * p<0.05

Intragastric pressure measurement

Effect of mirtazapine (15 mg) on proximal and distal IGP during intragastric nutrient infusion

During intragastric infusion of nutrient drink, the proximal IGP dropped from baseline, followed by a gradual recovery. After placebo treatment the nadir did not differ compared to baseline (baseline: -6.73±0.78 mm Hg vs. 3 weeks placebo: -5.84±0.86 mmHg, p=0.45), as did the time to reach nadir (baseline: 5.07±0.89 min and 3 weeks placebo: 5.40±0.58 min; p=0.73). The area above the curve (AAC) was also comparable to baseline (AAC baseline: -34.84±4.00 mmHg.min and AAC after 3 weeks placebo: -27.16±4.61 mmHg.min, p=0.22) (Figure 5.a.). Antral IGP (n=13) did not show any differences as well (AAC baseline: -17.62±3.23 mmHg.min and AAC after 3 weeks placebo: -12.52±3.24 mmHg.min, p=0.26).

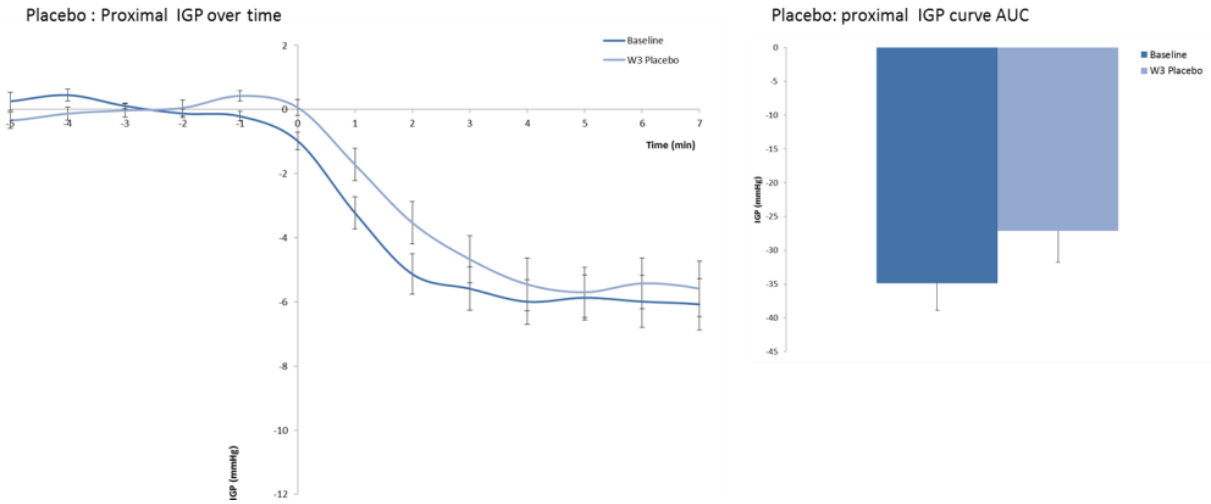


Figure 5.a: Intragastric pressure measurement after placebo. Intragastric infusion of a nutrient drink (300 Kcal) until maximal satiation started at time point 0. Before and after placebo treatment the

IGP drop from baseline did not differ significantly (AUC baseline: -34.84±4.00 mmHg and AUC after 3 weeks placebo: -27.16±4.61 mmHg, p=0.22).

During the meal, the IGP was significantly increased compared to baseline after 3 weeks of mirtazapine treatment. The nadir was found to be higher compared to baseline (baseline: -8.25±0.76 mmHg vs. mirtazapine: -5.97±0.49 mmHg, p=0.01), hence, the time to reach nadir was similar to baseline (baseline: 7.87±0.81 min vs. mirtazapine: 6.53±0.98 min; p=0.33) (Figure 5.b.). The AAC was also significantly decreased after mirtazapine treatment (AAC baseline: -43.26±4.52 mmHg.min vs. AAC mirtazapine: -28.89±3.06 mmHg.min, p=0.005) (Figure 5.b.). Antral IGP (n=15) did not show any differences after mirtazapine treatment (AAC baseline: -22.09±2.63 mmHg.min vs. mirtazapine: -16.88±2.38mmHg, p=0.23).

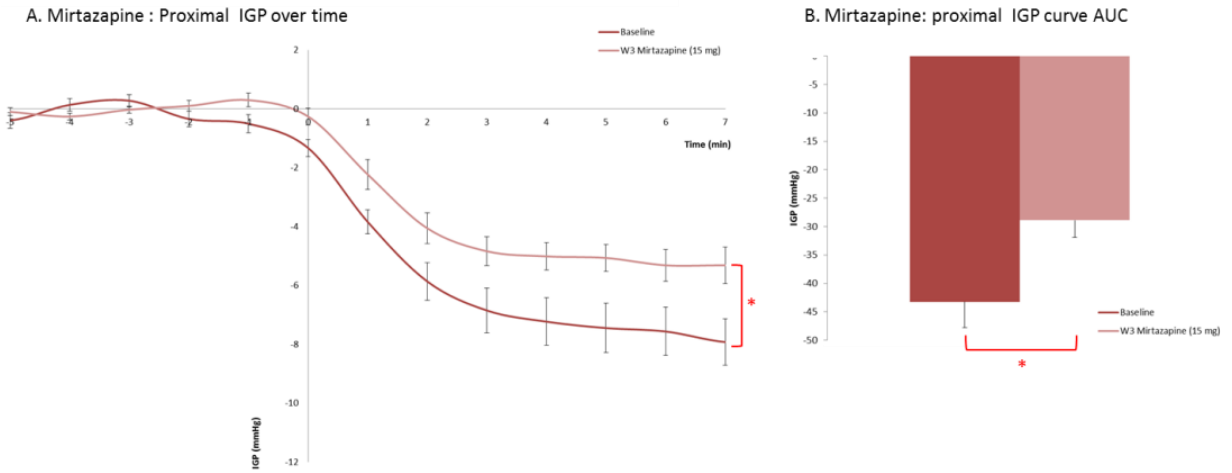


Figure 5.b: Intragastric pressure measurement after mirtazapine treatment. Intragastric infusion of a nutrient drink (300 Kcal) until maximal satiation started at time point 0. After 3 weeks of mirtazapine treatment, the IGP drop from baseline was significantly increased compared to baseline (AUC baseline: -43.26±4.52 mmHg and AUC 3 weeks mirtazapine: -28.89±3.06 mmHg, p=0.005). Moreover, the nadir was found to be higher compared to baseline (baseline: -8.25±0.76 mm Hg vs. 3 weeks mirtazapine: -5.97±0.49 mmHg, p=0.01).
**** p<0.05***

Effect of mirtazapine on satiation during intragastric infusion of ND

There was no difference in nutrient tolerance and time to reach maximal satiation after treatment with placebo compared to baseline. The mean maximal tolerated volume was 1110±130.18 Kcal (time to max satiation: 12.33±1.45 min) at baseline and 1020±152.30 Kcal after 3 weeks placebo (time to max satiation: 11.33±1.69 min) (p=0.27) (Figure 6.a.). After treatment with mirtazapine, no differences were found when compared to baseline (baseline: 1170±129.38 Kcal, time: 13±1.44 min and 3 weeks after treatment: 1104±133.62 Kcal, time: 12.27±1.48 min; p=0.42) (Figure 6.b.).

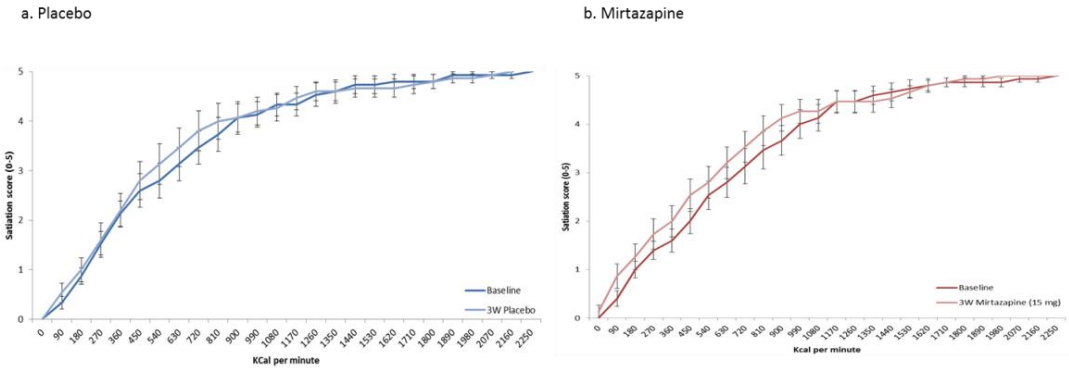


Figure 6: Satiation curve. There was no difference in nutrient tolerance and time to reach maximal satiation after treatment with placebo and mirtazapine compared to baseline. The mean maximal tolerated volume at the placebo group, was 1110±130.18 Kcal at baseline and 1020±152.30 Kcal after 3 weeks placebo (p=0.27). The mirtazapine group drank 1170±129.38 Kcal at baseline and 1104±133.623 Kcal after 3 weeks mirtazapine treatment (p=0.42).

Effect of mirtazapine on epigastric symptoms during the barostat and IGP measurement

After 3 weeks of mirtazapine treatment, during the gastric barostat measurements, area under the curve (AUC) of the VAS scores of hunger were increased compared to baseline (AUC baseline: 167.9±45.1, AUC after 3 weeks: 282±67.6; p=0.03). After the meal, upper abdominal bloating (AUC baseline: 325.6±81.8, AUC after 3 weeks: 182±58.7 p=0.06), postprandial fullness (AUC baseline: 443±84.6, AUC after 3 weeks: 239±61.9, p=0.06) and belching (AUC baseline: 162±53.9, AUC after 3 weeks: 58±18.2, p=0.08) tended to be decreased after mirtazapine treatment compared to baseline (Figure 7.a.).

Similar results were observed during the IGP measurements, the VAS scores of upper abdominal bloating (AUC baseline: 320±63.88, AUC after 3 weeks: 228±65.04, p=0.19), postprandial fullness (AUC baseline: 493±61.29, AUC after 3 weeks: 439±66.77, p=0.57), nausea (AUC baseline: 112±39.61, AUC after 3 weeks: 59±33.71, p=0.22) and belching (AUC baseline: 136±51.62, AUC after 3 weeks: 60±31.63, p=0.03) tended to be decreased compared to baseline after the meal (Figure 7.b.).

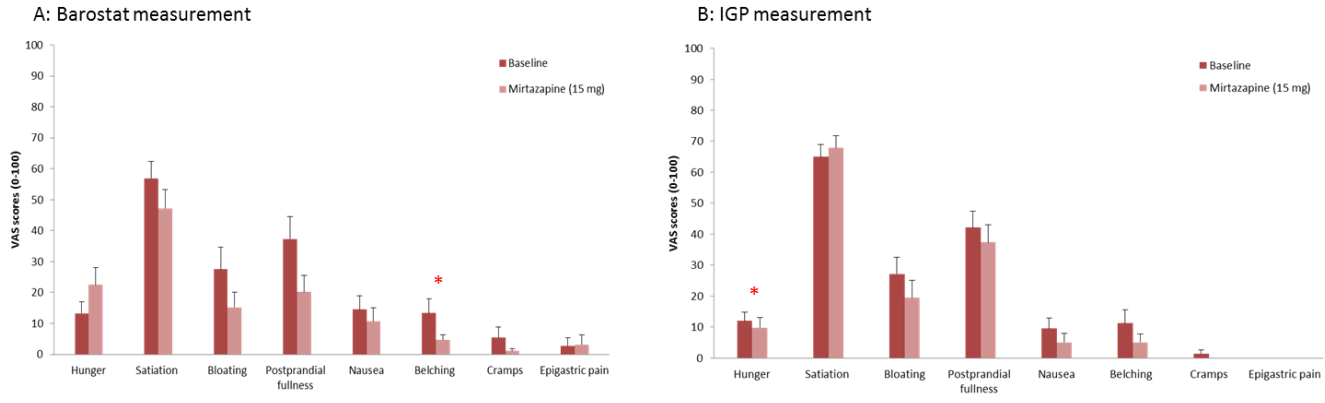


Figure 7. Mean VAS score after the meal in the mirtazapine group. The (a) barostat VAS scores and the (b) IGP VAS scores showed similar results. Both measurement show that mirtazapine tend to decrease symptoms such as upper abdominal bloating, postprandial fullness, nausea and belching compared to baseline. *p<0.05

5.3.4. Discussion

Previous studies have shown efficacy in FD patients with weight loss and potential efficacy in refractory gastroparesis (139, 140, 142, 143, 315-317). However, little is known about the effects of this drug on gastric sensorimotor function and, therefore, we aim to investigate this in healthy subjects. To do so, we used a classical gastric barostat study. This allows evaluate gastric sensitivity to isobaric balloon distention and gastric accommodation. However, this technique is very invasive and difficult to tolerate, and it has been previously shown that the intragastric balloon might affect the physiologic gastric responses (155, 158, 257). Hence, for the present study, we also measured the effect of mirtazapine on the intragastric pressure profile during nutrient infusion challenge with a HRM. The advantage of this technique is that the HRM probe is well tolerated, easy to perform and it is possible to assess simultaneous information about the IGP in the proximal and distal stomach, the gastric tone and the contractions in fasted and fed state, the gastric accommodation reflex as well as nutrient volume tolerance (160, 166, 258, 259).

In the study, blinded healthy subjects were instructed to take mirtazapine (15 mg) or placebo before bed time for 3 weeks. Gastric barostat and IGP measurements were planned before (baseline measurements) and at the end of 3 weeks of treatment. After 3 weeks of placebo, no significant results were observed on demographic features, gastric compliance, gastric sensitivity to distention, gastric accommodation and nutrient tolerance. These observations confirm the good reproducibility of the measurements separated by a period of 3 weeks (318).

After 3 weeks treatment with mirtazapine, the principal adverse event reported during this study was fatigue. The sedative effect of mirtazapine is already well-established in the literature and it is known that it tends to disappear during continued administrations after the first week of treatment (311, 312, 319). It has been suggested that the sedative effect of mirtazapine might be due to its H_1 receptor antagonistic properties (319). One HV was withdrawn from the study, due to urticaria during the treatment period with mirtazapine. The urticaria disappeared 3 days after the treatment with mirtazapine was stopped.

Our study showed that mirtazapine does not affect gastric compliance, but gastric sensitivity to distention tended to be decreased. In the literature, mirtazapine has been used as an analgesic to treat chronic tension-type headache and fibromyalgia and it was able to ameliorate visceral hypersensitivity and nerve injuries in animal models (314, 320-323). The antinociceptive function of mirtazapine has been associated with its combined effect through central serotonergic, noradrenergic and opioid receptors (321). The gastric accommodation measured with the gastric barostat showed no significant difference in meal-induced gastric accommodation. In contrast, the IGP measurements showed a significant decrease in drop of IGP from baseline during the intragastrically infused liquid meal, indicating a possibly decreased gastric accommodation. On the other hand, nutrient volume tolerance during the IGP measurement was not significantly altered. This effect is at variance with our previous observations that indicated impaired gastric accommodation or a reduced IGP drop to be associated with decreased nutrient tolerance (160, 166, 258, 259).

A placebo-controlled mirtazapine study in FD patients showed improved nutrient tolerance after 8 weeks of treatment (139). Unlike the healthy volunteers, the patients used mirtazapine after a period of weight loss, which may have enhanced food tolerance effects of the drug. Furthermore, we cannot exclude the possibility that a longer treatment period might have revealed an effect on nutrient intake in the present study. On the other hand, we found significant weight gain in the healthy subjects after

3 weeks and increased hunger ratings during the barostat measurement with mirtazapine, indicating that mirtazapine was already affecting pathways that drive increased body weight. Previous studies have implicated enhanced nutrient ingestion and leptin-mediated increased body fat mass in the weight gain effect of mirtazapine (311-313). Finally, symptom assessment during IGP and barostat measurements showed a tendency towards decreased upper abdominal bloating, postprandial fullness, nausea and belching compared to baseline after the meal. This is in line with previous studies that showed that mirtazapine may reduce nausea through its $5HT_3$ receptor antagonistic properties and, that it may improve other dyspepsia symptoms by enhancing the gastrointestinal transit (141, 142, 144, 315).

Taken together, the observations of the present healthy volunteer study show that mirtazapine does not display changes in gastric sensorimotor function that could explain its beneficial effects on FD symptoms and nutrient tolerance. The occurrence of weight gain and decreased meal-induced symptoms in spite of a suppressed meal-induced IGP drop, point towards a central mode of action.

Chapter 6

The evaluation of novel therapeutic options in FD patients

6.1.1. Introduction

Gastroparesis is defined as the presence of delayed gastric emptying in the absence of mechanical obstruction, and associated with symptoms of postprandial fullness, early satiety, nausea, vomiting and upper abdominal bloating (68). Gastroparesis can occur as a complication of diabetes mellitus, but in the majority of cases no underlying causes can be found and gastroparesis is defined as idiopathic (63). Gastroprokinetic drugs are considered the treatment of choice for gastroparesis, aiming at improving symptoms through stimulation of gastric motility and gastric emptying rate (222). However, a systematic analysis of prokinetic agent trials in idiopathic and diabetic gastroparesis to date failed to find a significant association between the improvement in emptying rate and symptomatic benefit (126). More recent attempts to develop novel prokinetic agents have focused on studies with ghrelin receptor agonist in diabetic gastroparesis, but no consistent symptomatic benefit has been observed (324-326).

5-HT4 receptor agonists are probably the best-studied class of agents for the treatment of gastroparesis (126). Prucalopride, a highly selective 5-HT4 receptor agonist, is approved for the treatment of chronic constipation with insufficient response to laxatives (288, 289). After oral administration, prucalopride is well absorbed in the gastrointestinal tract and it has an absolute bio-ability of more than 90% (287). The plasma half-life of prucalopride (2 mg) is 24 hours and it reaches the maximum serum concentration between 2 and 3 hours after intake (287). Furthermore, prucalopride has shown a favorable safety profile in studies and clinical practice and it does not affect the QT interval (115, 327). Prucalopride stimulates colonic transit, and this is the basis for its effectiveness in chronic constipation (283, 284). However, prucalopride was also shown to enhance gastric emptying in a dog model, in healthy volunteers and in patients with chronic constipation (284, 290, 305).

Our aim was to evaluate the efficacy of prucalopride in patients with idiopathic gastroparesis in a randomised, double blind cross-over study.

6.1.2. Materials and methods

Patients

Consecutive patients with symptoms suggestive of gastroparesis and with established delayed gastric emptying for solids (328) were eligible for this double-blind randomized cross-over study. Patients presented to the motility outpatient clinic because of symptoms suggestive of gastroparesis, and all underwent careful history taking and clinical examination, routine biochemistry, upper gastrointestinal endoscopy, upper abdominal ultrasound and a gastric emptying breath test (176, 328). Exclusion criteria were the presence of diabetes, reflux esophagitis grade B or higher, gastric atrophy or erosive gastroduodenal lesions on endoscopy, suspected small bowel obstruction, major abdominal surgery, underlying psychiatric illness, and the use of non-steroidal anti-inflammatory drugs or steroids.

Study protocol

An overview of the study design is shown in Figure 1. During a to-week run-in period, patients filled out daily diaries and underwent a gastric emptying breath test study (details outlined below). At the end of the run-in period, they filled out the PAGI-SYM questionnaire, which comprises the Gastroparesis Cardinal Symptom Index (GCSI), as well as the PAGI-QOL quality of life questionnaires

(details outlined below) (199, 214, 217, 329, 330). After the run-in period, patients entered a double-blind controlled treatment phase of 4 weeks with prucalopride 2 mg or matching placebo, taken in the morning. This was followed by a two-week washout period, and another 4-week double-blind controlled treatment period in which the patient was crossed over to the other treatment arm in a blinded fashion. Patients also used a diary to indicate the severity on 10 cm visual analogue scales for 8 epigastric symptoms (epigastric pain, postprandial fullness, upper abdominal bloating, early satiation, nausea, vomiting, belching, heartburn), as well as overall symptom assessment and the number of bowel movements and their consistency on the Bristol Stool Form Scale (331). The daily diary was filled out throughout the entire study period, and the gastric emptying test, PAGI-SYM/GCSI and PAGI-QUOL questionnaires were repeated at the end of each treatment period and at the end of the washout period.

All drugs potentially affecting gastrointestinal motility or sensitivity were discontinued at least one week prior to the start of the study. Informed consent was obtained from each participant. The protocol had been previously approved by the Ethics Committee of the University Hospital.

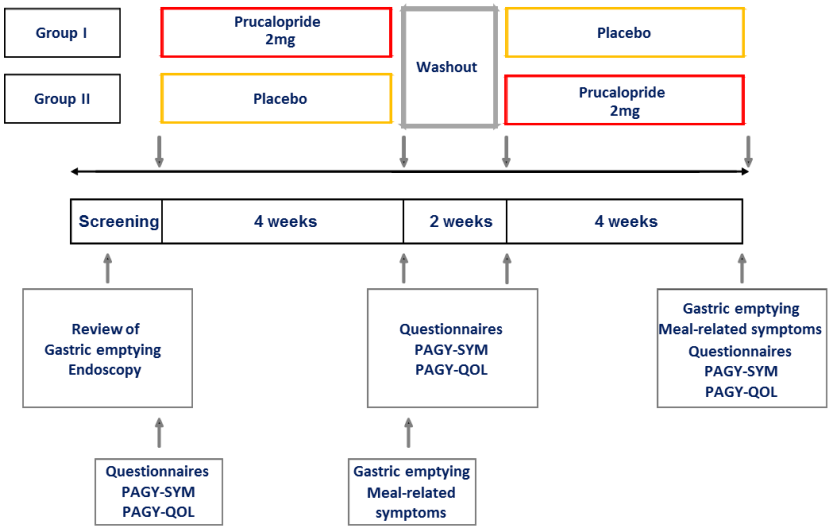


Figure 1. Schematic outline of the study.

Gastric emptying breath test and meal-related symptoms

Gastric emptying rates for solids and liquids were determined using the 14C octanoic acid and 13C glycin breath test (176, 328). The test meal consisted of 60 g of white bread, an egg, the yolk of which was doped with 74 kBq of 14C octanoid acid sodium salt (DuPont, NEN Research, Boston, MA, USA) and 300 ml of water in which 100 mg 13C glycin (99% enrichment; Isotec, Miamisburg, OH, USA) was dissolved. All meals were consumed within a five minute period. The total caloric value of the test meal was 250 kcal. Breath samples were taken before the meal and at 15-minute intervals for a period of 240 minutes postprandial. At each sampling point, the subject exhaled into two different containers for measuring exhaled 13C and 14C respectively. One was a liquid scintillation vial containing 2 ml of 1 M hyamine hydroxide and 2 ml of ethanol together with 1 drop of thymolphtalein solution. This amount of hyamine is neutralized by 2 mM of CO2. The end point of neutralization is indicated by discoloration of the indicator. After discoloration, 10 ml of scintillation cocktail (Hionic Fluor, Packard) was added and radiation was determined by liquid scintillation counting (Packard Tri-Carb Liquid

Scintillation Spectrometer, model 3375, Packard Instrument Company, Downers Grove, IL, USA). For 13C measurements breath was collected by blowing directly into a tube. The 13C breath content was determined by on-line gas chromatographic purification-isotope ratio mass spectrometry (ABCA, Europe Scientific, Crewe, UK). At each breath sampling, the patient was asked to grade the intensity (0-3; 0=absent, 1=mild: present in a non-bothersome intensity, 2=relevant: clearly present and bothersome but not of such intensity that it would interfere with normal daily activities and 3=severe: clearly present and of such intensity that it would interfere with normal daily activities) of six different symptoms (epigastric pain, bloating, postprandial fullness, nausea, belching and epigastric burning) (176, 328).

Patients Assessment of Gastrointestinal Symptoms (PAGI-SYM)

The self-reported PAGI-SYM questionnaire is composed of 20 items and 6 subscales: heartburn/regurgitation (7 items), nausea/vomiting (3 items), postprandial fullness/early satiety (4 items), bloating (2 items), upper abdominal pain (2 items), and lower abdominal pain (2 items). The severity of each symptom item over a 2-week recall period is scored from 0 (none or absent) to 5 (very severe) (214, 329).

Subscale scores for the PAGI-SYM are calculated by averaging across items comprising the subscale; scores vary from 0 (none or absent) to 5 (very severe).

Gastroparesis Cardinal Symptom Index (GCSI)

The GCSI score consists of the nausea/vomiting (3 items), postprandial fullness/early satiety (4 items) and bloating (2 items) domains of the PAGI-SYM. The severity of each subdomain is calculated as above. The total GCSI score was obtained by averaging the three symptom sub-scale scores (199, 217).

Patients Assessment of Gastrointestinal Quality of Life (PAGI-QOL)

The PAGI-QOL is a validated scale to assess quality of life in upper gastrointestinal disorders (330). The questionnaire uses 30 questions, measured on a 0-5 scale (none of the time to all of the time) to cover 5 domains (daily activities, clothing, diet and food habits, relationship, psychological well-being and distress).

Data analysis

The results of the 13CO2 and 14CO2 breath tests were expressed as the percentage 13CO2 and 14CO2 respectively, excreted per hour by calculating procedures described elsewhere. For both carbon labels, CO2 production was assumed 300 mmole/m2 of body surface per hour. Gastric half emptying time (t1/2) was calculated from the 13CO2 and 14CO2 excretion curves as previously described (176, 328). Solid gastric emptying was considered severely delayed if t1/2 was more than 109 min and liquid emptying was considered severely delayed if t1/2 was more than 75 min (176, 328). Symptom scores were obtained before and for 4 hours after the standardized meal. For each symptom, a meal-related severity score was obtained by adding scores at all time-points. A cumulative meal-related symptom score was obtained by adding individual symptom severity scores.

The questionnaires were scored as described above. Using the daily diaries, weekly mean severity scores for symptoms as well as stool consistency and stool frequency scores were calculated. In case of missing values, the last observation was carried forward for numerical analyses.

Statistical analysis

Data are presented as mean ± SEM. The primary outcome variable for this pilot trial was the GCSI score at the end of each treatment arm. Secondary outcome variables were the change in GCSI score from baseline, effects of treatment on the severity scales of the PAGI-SYM subscales, on solid and liquid gastric emptying rates, and on quality of life. The study was powered to detect a 30% difference in GCSI symptom scores with 85% sensitivity at a $p<0.05$.

6.1.3. Results

Patient characteristics

Twenty eight patients (7 men) with symptoms suggestive of gastroparesis and established severely delayed emptying of solids entered the study. The mean age was 42.3□4.6 years, and the BMI was 23.4±0.9 kg/cm2. Gastric half emptying times for solids and liquids were 141±17 and 92±26 minutes respectively.

Conduct of the study

Thirteen patients were randomized to receive prucalopride first and 15 were randomized to receive placebo first. Baseline characteristics are summarized in Table 1 and were similar in both groups.

Table 1. Characteristics of both patient groups.

	Idiopathic, prucalopride first	Idiopathic, placebo first	P
Female / male	9 / 4	12 / 3	NS
Age	39.5±3.T	44.8±3.7	NS
BMI	22.9±1.2	25.8±1.4	NS
Solid emptying t1/2 (min)	155±25	129±17	NS
Liquid emptying t1/2 (min)	104±25	91±30	NS
GCSI	2,84±0.81	2,54±0,29	NS

Six patients dropped out from the study (Figure 2). In the placebo first group, one subject was lost to follow-up after the screening period and one stopped participation because of nausea during the first treatment phase. In the prucalopride first group; one subject had a major adverse event (small intestinal volvulus during the first treatment phase), one subject stopped because of diarrhea and one because of headache during the first treatment phase; two subjects were lost to follow-up after the screening period. All other patients participated in the full study protocol as planned.

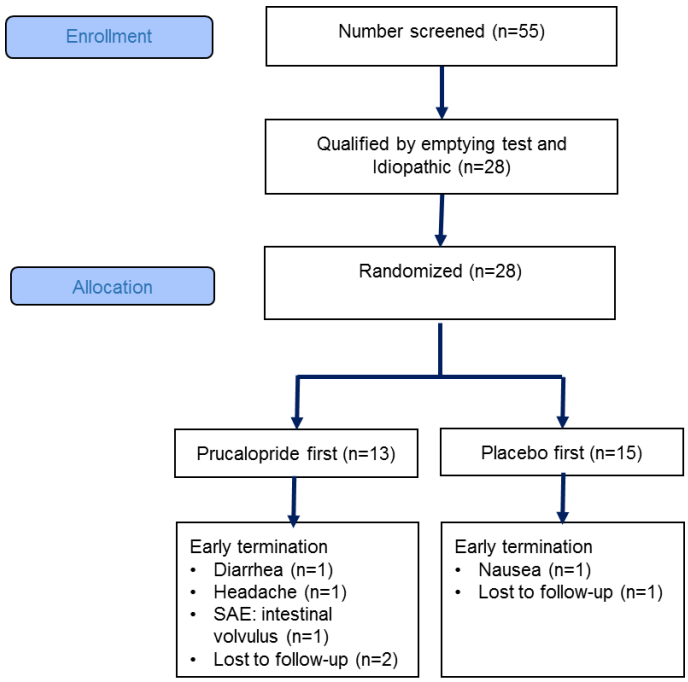


Figure 2. Patient flow diagram.

Run-in versus washout period

To assess the possibility of a carry-over effect, symptom severity scores at the end of the run-in phase and the washout period were compared. No significant differences were found for any of the GCSI and PAGI-SYM subscales between both time points (nausea/vomiting 1.75±0.29 vs.1.67±0.27; fullness/satiation 3.30±0.21 vs. 2.98±0.27; bloating/distention 3.14±0.31 vs. 2.52±0.34; pain/discomfort 2.90±0.21 vs. 2.45±0.30; lower abdominal pain 1.60±0.29 vs. 1.62±0.27 and reflux 2.01±0.25 vs. 1.65±0.26). These symptom similarities argue against a carry-over effect and allow a pooled analysis of the active vs. placebo treatment period, regardless of the treatment order.

Symptom pattern

After 4 weeks of prucalopride treatment, the GCSI (prucalopride 1.61±0.22 vs. 2.40±0.20 during placebo and 2.73±0.18 at baseline, both $p<0.0001$) and its subscales of nausea/vomiting, fullness/satiation and bloating/distension were significantly better compared to placebo treatment and compared to baseline (Figure 3). In addition, the PAGI-SYM subscale of reflux was significantly lower during prucalopride compared to placebo treatment, and the subscales of abdominal pain/discomfort and reflux were significantly better during placebo treatment compared to baseline (Figure 3).

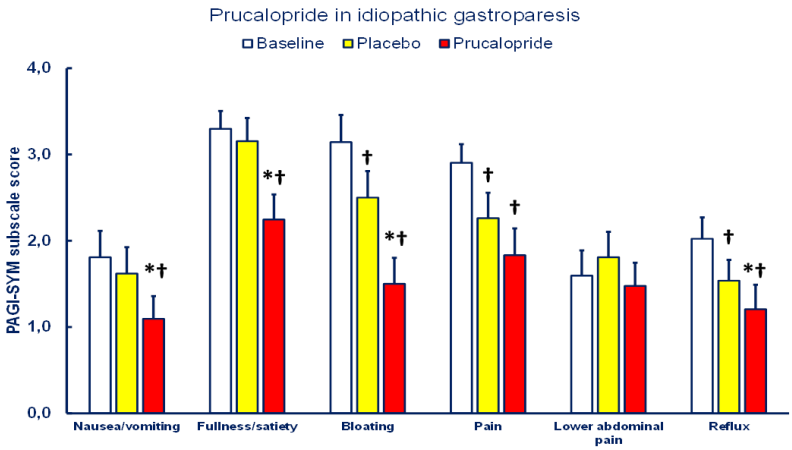


Figure 3. Influence of prucalopride versus placebo on subscales of the PAGI-SYM questionnaire. * p<0.05 compared to placebo arm; † p<0.05 compared to baseline.

Gastric emptying rate

After 4 weeks of prucalopride treatment, solid and liquid half emptying times were 86±13 and 73±4 min respectively. Solid, but not liquid half emptying times with prucalopride were significantly shorter than during placebo treatment (respectively 129±20 min, p=0.0067 and 87±16 min, NS) or at baseline (respectively 141±17 min, p=0.0003 and 92±26 min, NS).

Quality of Life

After 4 weeks of prucalopride treatment, the PAGI-QOL (prucalopride 1.20±0.26 vs. 1.55±0.33 during placebo and 1.90±0.39 at baseline, both p<0.01) and its subscales of clothing and diet were significantly better compared to placebo and compared to run-in (Figure 4). In addition, all PAGI-QOL subscales except for psychological well-being were significantly better during prucalopride treatment compared to baseline. Furthermore, the total PAGI-QOL score and the subscales of daily activities and relationships were significantly lower during placebo treatment compared to baseline (Figure 4).

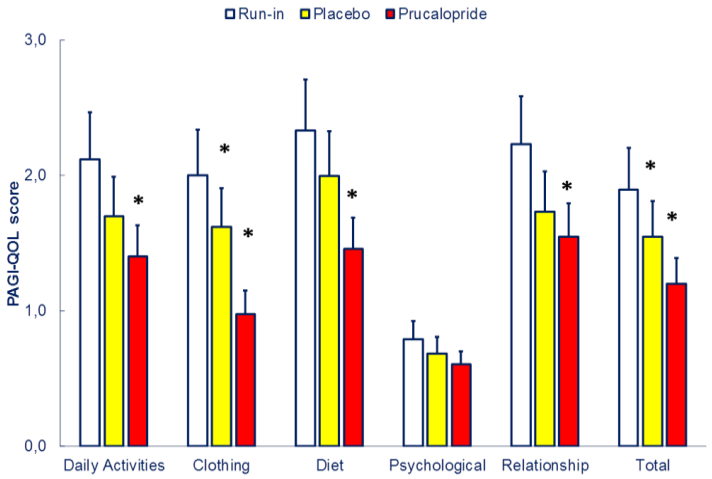


Figure 4. Influence of prucalopride versus placebo on subscales of the PAGI-QOL questionnaire. * p<0.05 compared to placebo arm.

Daily diaries

Daily diaries confirmed significant improvement compared to placebo treatment of symptom severity ratings for abdominal pain, postprandial fullness, bloating, early satiation, nausea and overall symptom severity. The same symptoms, as well as belching, were significantly improved compared to baseline during prucalopride treatment (details not shown).

The number of bowel movements per day rose from 1.20±0.06 at baseline to 1.50±0.08 during the first 2 weeks of prucalopride therapy ($p=0.004$), to normalize back to 1.29±0.08 during the second 2 weeks of prucalopride treatment (NS). The proportion of Type 1 and 2 bowel movements during prucalopride treatment (8%) was significantly lower compared to placebo or to baseline (respectively 22 and 13%, both $p<0.05$). However, there was no correlation between the change in symptom pattern (frequency or consistency) and the change in symptom pattern.

Adverse events

One serious adverse event occurred: one patient developed intestinal volvulus 18 days after start of treatment with prucalopride. Adverse events leading to termination were one case of diarrhea and one of headache during prucalopride treatment and a case of nausea during placebo treatment.

Transient diarrhea was reported by 9 patients during prucalopride treatment, lasting 1-7 days, and in 1 patient during placebo treatment. Transient headache was reported by 8 patients during prucalopride treatment, lasting 1-7 days and in one patient during placebo treatment. Abdominal cramps were reported during prucalopride and placebo treatment by one patient each. Cystitis occurred in one patient during each treatment, respiratory infection in one patient during prucalopride treatment.

6.1.4. Discussion

In this study, we evaluated the efficacy of the selective 5-HT4 agonist prucalopride in a placebo-controlled cross-over trial in patients with idiopathic gastroparesis. Prucalopride treatment improved symptoms as assessed by the GCSI compared to placebo and to baseline. The beneficial effect of prucalopride was present for all three subscales of the GCSI: nausea/vomiting, fullness/satiety and bloating/distention. In line with the pharmacodynamics properties of 5-HT4 agonists, prucalopride treatment was also associated with improved solid gastric emptying rate compared to placebo and baseline. However, there was no correlation between the symptomatic benefit of prucalopride treatment and the enhancement of gastric emptying rate. Prucalopride also improved upper abdominal pain and reflux symptoms, assessed by the PAGI-SYM questionnaire. Finally, prucalopride improved overall quality of life and the subscales of daily activities, clothing, diet and relationship.

From its use in chronic constipation, prucalopride is known to be associated with adverse events of diarrhea, headache and nausea (115, 287, 327). The same adverse events occurred more frequently in the prucalopride arm in the present gastroparesis trial, and led to a slightly higher discontinuation rate during prucalopride treatment. However, most of the adverse events of diarrhea and headache were transient.

The efficacy of prucalopride as observed in this idiopathic gastroparesis trial raises the question whether the drug would also be efficacious in functional dyspepsia/postprandial distress syndrome patients with normal gastric emptying (41, 332, 333). The lack of a correlation between the symptomatic benefit of prucalopride and the change in gastric emptying rate suggests that enhanced emptying is not the mechanism underlying the symptomatic beneficial effect. On the other hand, in

healthy volunteers we demonstrated that prucalopride has an inhibitory effect on gastric accommodation and sensitizes the stomach to gastric distention (334). As hypersensitivity to gastric distention and impaired accommodation are key mechanisms implicated in symptom generation in functional dyspepsia, it is conceivable that delayed gastric emptying is a marker of a subgroup of patients that may respond to the strong motility stimulatory effects of the selective 5-HT4 agonist prucalopride (21, 190). On the other hand, a study exploring the effects of prucalopride in FD/PDS patients with normal gastric emptying should be considered.

Although 5-HT4 agonists are often considered a preferred pharmacological class of prokinetic drugs, several recent studies failed to show significant benefit (63, 68, 126, 222, 335). Most recently, the focus has been on ghrelin agonists, but also these studies have failed to show consistently beneficial effects (292, 324-326). Most of the recent studies have focused on diabetic gastroparesis (126, 222, 324-326, 335), while the present study included idiopathic gastroparesis patients. It is conceivable that the selection of idiopathic gastroparesis patients, where sensory neuropathy is not an issue, favored a better symptom assessment compared to diabetic gastroparesis, where neuropathy may confound symptom assessment (63, 68, 332, 333). Larger scale studies with prucalopride in both diabetic and idiopathic gastroparesis may clarify this possibility.

The current study has a number of limitations. We recruited a small group of patients with idiopathic gastroparesis in a tertiary care center. The findings are not necessarily applicable to patients seen at other levels of care, and patients with organic or drug-induced causes of gastroparesis. Treatment duration, only 4 weeks, was also short, and the cross-over design is another limitation. On the other hand, the cross-over design allowed a more accurate evaluation of changes in emptying rate and symptom pattern across treatment arms, and the analysis of symptoms during the treatment-free interval showed no signs of a carry-over effect. Finally, we evaluated only one dose of prucalopride, chosen for its use in chronic constipation, but it is unclear whether this is the optimal dosing for gastroparesis.

In summary, this single-center crossover study of prucalopride showed symptomatic benefit of the drug in idiopathic gastroparesis. These encouraging findings should preferably be studied in a larger, multi-setting, parallel-group design study, and prucalopride’s efficacy in postprandial distress syndrome without delayed emptying also merits studying.

Chapter 7

General discussion and future prospects

Functional dyspepsia (FD) is defined by the Rome consensus as the presence of one or more dyspeptic symptoms in the absence of any organic or metabolic disease that is likely to explain the symptoms (41, 42). Functional dyspepsia is a multifactorial syndrome and its pathophysiological background is still incompletely understood.

Over the last years, different pathophysiological mechanisms including gastric motor and sensory dysfunction, have been suggested to play a key role in FD (16). However, the association of the different underlying mechanisms to the varied clinical symptom presentation in FD patients has yet to be fully elucidated. Taking into account such limitations, and in order to support FD patient management, the Rome consensus proposed to classify FD patients into postprandial distress syndrome (PDS), characterized by meal-related symptoms, and epigastric distress syndrome (EPS), characterized by meal-unrelated symptoms (42, 197). However, this guideline was mostly based on expert opinion and population factor analysis. In clinical practice, a major overlap between PDS and EPS is found, negatively affecting the usefulness of this subdivision (42). Furthermore, from this subdivision it has been suggested that motor dysfunctions such as impaired gastric accommodation (present in 40% of the FD population) and delayed gastric emptying time (present in 35% of the FD population) might be more common in the PDS group while sensitivity dysfunctions such as hypersensitivity to gastric distention (present in 30% of the FD population) might be more common to the EPS subgroup (16, 22, 36, 60, 61). Nevertheless, this has never been extensively evaluated. In **chapter 2.1** we showed that the prevalence of gastric hypersensitivity, impaired gastric accommodation and delayed gastric emptying were similar in the different FD subgroups (PDS, EPS and the overlap PDS-EPS subgroup). The PDS subgroup resembled clinically more the overlap subgroup showing significantly decreased BMI, probably corresponding to the decrease caloric intake in this population due to meal-related symptoms, and increased occurrence of abdominal bloating and nausea. The latter was also associated with delayed gastric emptying time in the overlap subgroup. Nevertheless, in **chapter 2.3** we showed that the severity of symptoms was inconsistently and poorly associated with the gastric emptying rate after a standardized meal in FD as well as in the different subgroups, suggesting that delayed gastric emptying is not the primary cause leading to symptoms. Finally, while the restricted number of EPS patients that underwent a gastric barostat test limited the interpretation of the results, in **chapter 2.1**, in **chapter 2.3** we observed that the EPS subgroup is noticeably different from the PDS and the overlap subgroups, confirming the lack of relationship of pure EPS symptoms to the meal and suggesting a different pathophysiological background for EPS symptom generation. From these results is clear that the pathophysiology of FD is complex and continues to require further studies. The suggested pathophysiological abnormalities were present in a subset of patients, also observed in previous studies, but their presence and severity was not related to the Rome subdivision of FD. In this study only 3 pathophysiological mechanisms were assessed, while it is known that FD is multifactorial. Other mechanisms, e.g. duodenal mucosal alterations and duodenal hypersensitivity to acid or lipid, were not taken into account. Moreover, the overlap group included more than 50% of the population and the EPS subgroup was rather small, hampering decisive comparisons between groups. Nevertheless, these data show dissociation between the EPS on one hand and the PDS and the overlap subgroups on the other hand.

The relationship of symptoms to the ingested meal seems to gain increasing interest and **chapter 2.2** and **2.3** as well as previous experimental observations (43, 78, 99, 163, 190, 191, 195) showed its relevance. In **chapter 2.2.,** we described that a high proportion of non-PDS symptoms (epigastric pain

and nausea) are triggered or aggravated by the meal in the overlapping EPS-PDS group, compared to the pure EPS group. Redistribution of patients in whom epigastric pain and nausea were almost exclusively occurring postprandial into a "new" PDS group, significantly decreased the overlap between the "new PDS" and EPS groups. Further elaboration of this work in an experimental set-up in **chapter 2.3.,** also showed that that the reported frequency of meal-related symptoms in the PDS and the overlap PDS-EPS group correlated well with the meal-related symptom intensities scored after ingestion of the standardized meal. When again redistributing patients with non-PDS symptoms to the "new PDS" subgroup, this was supportive of the hypothesis that symptoms in the "adapted" PDS population were originating from the stomach, while symptoms in EPS and in the "adapted" overlap population may originate from the duodenum as previously suggested by Vanheel *et al* (191).

The management and treatment of FD patients relies on the facilitation and reduction of symptoms through corrections of altered sensorimotor function (96). Based on the symptom pattern, the proposed first-line pharmacotherapy choices for the EPS patients are antisecretory drugs, and more particularly proton pump inhibitors (PPIs) (96). Prokinetics are considered to be more effective in PDS patients and, as our data show that many overlap patients are clinically similar to PDS, these agents might also be of interest as first-line approach in the overlap subgroup (96, 222). However, the number and availability of prokinetic agents is limited and several recent attempts at developing novel prokinetics for FD have been unsuccessful (96, 119, 132, 222, 238, 239). Potential underlying reasons are the difficulties in selecting the right patient population (lack of accurate symptom assessment, overlap between PDS and EPS groups, pathophysiological heterogeneity) and the use of inappropriate endpoints and endpoint questionnaires in clinical trials. In **chapter 3** we describe the development and validation of a new Patient-Reported Outcome (PRO) instrument, the Leuven Postprandial Distress Scale (LPDS), for the assessment of symptoms and their responsiveness to treatment in patients suffering from PDS. In **chapter 3.2.**, by means of focus group sessions and cognitive interviews in PDS patients, the most relevant PDS symptoms were identified and used to generate item questions. To assess the individual symptoms, and based on patient preference, the severity of the symptoms was rated on a 0-4 severity scale with verbal descriptors and emoticons. Previously, we described that for PDS patients, both the severity and frequency of symptoms can be assessed and both are equally informative (**Chapter 3.1.**). Next, in line with regulatory guidance, we assessed and confirmed the consistency, reliability and sensitivity to detect symptom fluctuations and treatment outcomes of the LPDS questionnaire in the framework of a still blinded controlled parallel group treatment trial with itopride (a dopamine D2-receptor antagonist and cholinesterase inhibitor) in PDS patients recruited from 11 gastroenterology centers in Belgium (**chapter 3.3**). In a larger patient cohort, the performance and accuracy of the instrument was confirmed for both the "pure" PDS and the overlap PDS-EPS subgroups (**chapter 3.4**). This observation is again supportive of the similarities between the PDS and the overlap patients and, most importantly, it expands the usefulness of the instrument within the FD population. Only the pure EPS population, which is much smaller than the other groups, still lacks a validated endpoint instrument. Finally, the European Medicines (EMA) expressed its support to the use of the LPDS in clinical trials of patients with PDS and the Dutch LPDS instrument has already undergone validated translation into five languages. However, cognitive interviews of PDS patients from each country and their evaluation are still necessary to confirm the linguistic validation.

A more objective way to diagnose patients or to measure treatment efficacy would be to assess presence of and changes in a known pathophysiological mechanism such as impaired gastric

accommodation. The gold standard for measuring accommodation, the gastric barostat study, is invasive, difficult to apply in clinical practice and to date not successfully applied in multi-center studies (79, 158, 257). Recently, we proposed the measurement of intragastric pressure by a high resolution manometry catheter as a novel approach to study gastric accommodation (160, 258). The technique uses an identical catheter as esophageal manometry and therefore, it has the potential to gain a similar acceptance and feasibility level as an esophageal manometry. Studies on the IGP measurements during a nutrient tolerance test have shown its sensitivity to measure changes possibly associated to the gastric accommodation reflex (160, 166, 258, 259). The validity of this new tool could be shown by assessing differences between a control and a disease (e.g. FD patients) or by assessing predictable changes caused by a pharmacological agent. In **chapter 4.1** we showed clear differences between FD patients and HVs when measuring the IGP during the intragastric infusion of a liquid meal. The IGP drop was significantly decreased in patients and this was associated to a decreased nutrient tolerance. Such association between changes in IGP drop and nutrient volume tolerance has also been observed in response to pharmacological or mechanical interventions in healthy subjects (160, 258). In the present study, we also observed that 45% of the FD patients were classified as having an abnormal IGP response to the liquid meal. These results are in agreement with previous barostat studies that showed that a subgroup of FD patients presented decreased barostat balloon volumes after ingestion of 200 ml of a liquid nutrient meal (22, 36, 138). Piessevaux et al. linked the symptom pattern in FD patients to the intra-gastric distribution of a meal as assessed on radioscintigraphy images (195). These studies showed that proximal retention and early distal redistribution of a meal could be related to different symptoms and probably represented different pathophysiological mechanisms. Based on this information, we compared scintigraphy images of the gastric distribution of a liquid meal to simultaneous IGP measurements in FD patients and healthy controls (chapter 4.2). In this study again, a relationship was found between a decreased proximal IGP drop and a decreased nutrient tolerance. Moreover, the recovery of IGP from nadir was correlated to the increasing meal-induced satiation scores. Scintigraphy images showed that the liquid meal intragastric content preferentially accumulates in the proximal part of the stomach. The filling of the proximal stomach correlated better to satiation scores than the filling of the distal stomach. Furthermore, the area above the proximal stomach IGP curve correlated well with the area representing the proximal and the distal gastric volume accumulation until maximal satiation in all subjects. Similar correlations were not found for distal IGP values indicating the key role of the proximal stomach IGP in determining the distribution of the meal. Finally, the residual gastric content was high as only about 10% of the liquid was emptied into the duodenum during the study. However, the emptying rate in FD patients seemed to be slowed than in healthy subjects. The residual duodenal volume was also correlated to the IGP values, indicating that the drop in IGP may also affect gastric emptying time. However, it should be mentioned as a limitation that the control groups in these validation studies were significantly younger than the FD patients. Future studies should aim at including matched controls.

Pharmacological studies have shown some contradictory results when findings with the gastric barostat and with IGP measurements were compared. Previously, gastric barostat studies have shown that sildenafil enhanced gastric accommodation in healthy subjects (272). In contrast, our present study (**chapter 5.1**) showed that the IGP drop and nutrient tolerance were decreased after sildenafil suggesting an inhibitory effect on GA. Furthermore, GE rate for solids was delayed. Literature reports of the occurrence of dyspeptic symptoms after sildenafil intake are more conceivable with our IGP

observations than those during the gastric barostat test. The decreased nutrient tolerance shows better agreement with the IGP results than with the barostat results reported after sildenafil. Finally, both impaired GA and delayed GE are well-established pathophysiological mechanisms in FD and associated with postprandial fullness, nausea, decreased nutrient tolerance and weight loss. Mirtazapine was recently shown to provide symptom relief, increased nutrient tolerance and recovery of body weight in FD patients with weight loss (139). In a placebo-controlled mechanistic IGP study, 3 weeks of mirtazapine treatment significantly increased body weight in HVs, indicating that mirtazapine might affect pathways that drive increased body weight such as enhanced nutrient ingestion and leptin-mediated increased body fat mass (**chapter 5.2**). However, even though no important alterations were observed with the gastric barostat, the IGP drop was clearly decreased, indicating decreased gastric accommodation, and predicting decreased nutrient tolerance. However, in contract with our previous observations, in this case nutrient tolerance was not altered. In FD patients, mirtazapine showed improved nutrient tolerance after 8 weeks of treatment, suggesting that a longer treatment period might have revealed an effect on nutrient intake in the present study (139). Alternatively, the central effects of mirtazapine may also overcome the inhibitory effect of GA in the periphery. Finally, in **chapter 5.3**, no overall significant effect on IGP and nutrient tolerance was observed after prucalopride treatment in healthy subjects. Moreover, increased abdominal cramps were associated with increased antral IGP fluctuations reflecting enhanced antral contractile activity that may underlie enhancement of gastric emptying. The results of the barostat study were in fact difficult to adequately interpret. The study showed only a tendency to increase gastric sensitivity and postprandial gastric volumes. However, the latter may well be a reflection of nausea-related gastric relaxation, induced by prucalopride in the presence of a distending barostat bag in the stomach, rather than a true effect of prucalopride on the proximal stomach of the subjects. The absence of a major effect in the IGP studies and on nutrient volume tolerance are in agreement with this interpretation. In concordance with these results, symptom improvement was observed in FD patients with severe delayed gastric emptying after treatment with prucalopride, again confirming its gastroprokinetic properties and the symptom benefit that can be derived from enhancing gastric motility (**chapter 6.2**). Taken together, these results identify major limitations of the gastric barostat as a drug development or clinical tool, and suggest that IGP measurement during intragastric nutrient infusion may provide a good and less invasive alternative to measure gastric accommodation and nutrient tolerance in FD patients.

Taking into account the results obtained in the research conducted for this thesis, we were able to further characterized FD PDS, EPS and PDS-EPS overlap subgroups and to suggest a method to improve their subdivision. Future studies will need to evaluate whether the use of this adapted subdivision improves clinical management and especially treatment outcomes. In addition, we developed and validated a new PRO questionnaire to assess treatment outcome in clinical trials for PDS (with or without co-existing EPS). The availability of a state-of-the-art endpoint instrument should allow more therapeutic trials in FD and may facilitate the development of novel treatment approaches. As a tool to study relevant pathophysiology underlying FD symptoms, we and we proposed and validated the IGP measurement with the HRM as a minimally invasive approach for measuring gastric accommodation and nutrient tolerance. The method, used in healthy controls and patients, can also be used to evaluate effects of drugs, such as prokinetics or fundus relaxants, on gastric sensorimotor function and

nutrient tolerance. Using this approach, we showed that the prokinetic 5-HT4 agonist prucalopride stimulates antral phasic contractility, but sensitizes the stomach to distention. In a controlled cross-over study in FD patients with severely delayed emptying, prucalopride was superior to placebo in improving symptoms and gastric emptying. Future studies will be needed to address whether prucalopride is beneficial in FD patients without delayed gastric emptying.

In conclusion, FD is a complex disorder for which many questions still remain unanswered. Refinements of dyspepsia symptom evaluation and classification are still needed to appraise its heterogeneous phenotype in clinical practice. Dealing with FD remains a challenge for patients as well for clinicians and due to its multifactorial character, more extensive pathophysiological studies should be encouraged.

In future research, further validation of IGP measurements in FD patients and controls should be performed. These include comparison of results obtained with gastric manometry and the gastric barostat in the same patient population. Furthermore, we would like to explore the role of the duodenum in the control of gastric accommodation and IGP. This could be done by preventing nutrients entering the duodenum by using a small balloon positioned to block the pyloric region. Also, it would be interesting to study the autonomic responses (vagal activity) to a meal and their association to gastric accommodation and nutrient tolerance measured by IGP and/or the gastric barostat. Finally, the application of IGP measurements in different populations and its relation to nutrient tolerance and meal-related symptoms might be interesting to expand the usefulness in other functional gastrointestinal disorders and in disorders of food intake.

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Abstract

Functional dyspepsia (FD), defined as by the presence of one or more dyspeptic symptoms in the absence of any organic or metabolic disease that is likely to explain the symptoms, is one of the most common gastrointestinal disorders. For clinical management, it was proposed to subdivide FD into postprandial distress syndrome (PDS), characterized by meal-related symptoms and epigastric distress syndrome (EPS), characterized by meal-unrelated symptoms. Three major problems were addressed in this thesis. First, the major overlap between PDS and EPS that affects the usefulness of this subdivision, second, the lack of validated endpoint questionnaires to assess treatment efficacy in FD, and third, the assessment of impaired gastric accommodation (GA) as a relevant pathophysiological marker and therapeutic target in FD patients. The studies in this thesis show that the EPS subgroup is a clearly distinct entity, suggesting a different pathophysiological background for EPS symptom generation, while the PDS/EPS overlap and PDS subgroup resemble each other closely, suggesting that the meal is an important trigger in the origination of symptoms in PDS and overlap patients. Taking into account the relationship of symptoms to the meal helped to decrease the overlap group and increased the PDS group. Furthermore, we developed and validated a new patient-reported outcome instrument, the Leuven Postprandial Distress Scale (LPDS), for the assessment of symptoms and their responsiveness to treatment in patients suffering from PDS and PDS/EPS overlap. We also validated the intragastric pressure (IGP) measurement with intragastric nutrient infusion as a suitable and attractive tool to assess GA in health and FD. Finally, in a controlled cross-over trial, we showed that the prokinetic drug prucalopride, a 5-HT4 agonist, provides symptom improvement in FD patients with delayed gastric emptying. In conclusion, FD is a complex disorder in which the ingestion of a meal plays an important role in the triggering symptoms in the majority of patients. Impaired GA is a major pathophysiological mechanism which can now be assessed by IGP measurement. In FD patients with delayed emptying, the prokinetic agent prucalopride sees an effective treatment, although additional larger studies are warranted.

Summary

Functional dyspepsia (FD) is a highly prevalent gastrointestinal (GI) disorder defined as "the presence of epigastric symptoms in the absence of any underlying organic or metabolic disease that is likely to explain the symptoms". Based mainly on expert opinion, the Rome III consensus, proposed to subdivide FD into Postprandial distress syndrome (PDS), the major group, characterized by frequent postprandial fullness and/or early satiation, and epigastric pain syndrome (EPS), characterized by frequent epigastric pain and/or epigastric burning. In clinic samples, overlap of PDS and EPS is found in up to 50% of the patients, having a significant negative impact on the usefulness of the subdivision. The current therapeutic approach to this condition is based on the reduction of GI symptoms by means of diet, and especially medication such as acid suppressive and prokinetic drugs. Nevertheless, the efficacy of these therapies is limited and the development of novel options is hampered on the one hand by incorrect patient selection and inappropriate use of endpoints or endpoint questionnaires, and on the other hand by the heterogeneity and multifactorial nature of this condition. One of the most relevant proposed underlying mechanisms is impaired gastric accommodation (GA). GA is measured by means of the gastric barostat which is also considered the gold standard. However, this procedure is very invasive, difficult to tolerate and is likely to disturb normal physiological mechanisms. Hence, there is clearly a lack of a suitable measurement technique to optimally study and completely understand GA. Taking into account the gaps in the state of the art, this thesis comprises different objectives. The first objective was to optimize the assessment of the symptom pattern in FD and to explore its correlation with the underlying pathophysiology. Adequate symptom assessment is crucial for improving FD management and patient selection for clinical trials. On the one hand, this was done by exploring an approach to improve the subdivision and classification of FD into EPS and PDS subgroups and by studying the link between FD symptoms and the prevalence of several underlying pathophysiological mechanisms (chapter 2). On the other hand, we aimed to develop and validate an endpoint questionnaire for PDS based on the US FDA guidance (chapter 3). The second objective was to validate and apply a new technique to assess GA in response to food intake, the intragastric pressure (IGP) measurement by means of high resolution manometry. This validation includes comparing physiological measurements in FD and healthy controls (chapter 4), as well as assessment of pharmacological influences in healthy subjects (chapter 5) to explore the physiological control mechanisms of IGP and their role in generating dyspeptic symptoms. Finally, we aimed at expanding the therapeutic abilities in FD by conducting therapeutic intervention studies (chapter 5&6).
Chapter 2: FD patients fulfilling the ROME III criteria were subdivided into "pure" PDS, "pure" EPS and overlapping EPS-PDS subgroups. In addition to the classical Rome III questionnaire, the postprandial nature of a number of non-PDS symptoms was also assessed by a number of questions. 2.1: No differences in the prevalence of pathophysiological measured by the gastric barostat (impaired gastric accommodation, increased gastric sensitivity to distention) and by the gastric empting test were observed in the PDS, EPS and overlap subgroups as defined by the Rome III criteria. Despite the fact that there were no relations found between pathophysiology and symptoms, it is known that the meal is an important factor originating or aggravating symptoms in FD patients. 2.2: Compared to "pure" EPS patients, the overlapping EPS-PDS patients were characterized by a higher postprandial occurrence of non-PDS symptoms such as pain or nausea. Taking into account the prevalence of these symptoms in the overlap subgroup, patients were reclassified in the "new PDS" group. In this "adapted" subdivision the overlap PDS-EPS subgroup was shown to be reduced and the "new" PDS subgroup was

characterized by meal-related PDS symptoms as well as postprandial EPS symptoms. 2.3: Similar results were achieved in an experimental set-up where the frequency of symptoms (Rome III) and additional meal-related questions were compared to severity scores reported after the ingestion of a standardized meal. Here, it was shown that PDS and the overlap subgroup patients suffer similarly from symptoms after the meal, while the patients in the EPS subgroup, show a completely different symptom pattern.

Chapter 3: The choice to start with the PDS subgroup for the development of a new patient reported outcome (PRO) questionnaire, was driven by the larger proportion of PDS patients compared to EPS patients and by the availability of a large number of prokinetic drugs that need to be studied in this patient group. 3.1: By means of focus group sessions and cognitive interviews, relevant PDS symptoms were identified and question items were developed and expressed as questions as a pilot PRO instrument with a 5 point-severity-scale. 3.2: In order to assess its validity, reliability and responsiveness, a double blind, multicentre randomized, placebo-controlled parallel-group study with itopride in 60 PDS patients was conducted. During 2 week eligibility screening period and 8 weeks of treatment patients assessed the severity of their symptoms using the pilot LPDS questionnaire as daily diary. In addition, patients also filled out the Patient Assessment of Gastrointestinal Symptoms (PAGI-SYM), the Nepean Dyspepsia Index (NDI), overall treatment evaluation (OTE) and overall symptom severity (OSS) questionnaires. Construct validity was evaluated by known-group analyses and by correlating (changes in) LPDS scores with (changes in) anchors like OTE, OSS, early satiation/postprandial fullness domain of PAGI-SYM and eat/drink domain of NDI. The minimum Clinically Important Difference (MCID) was determined from clinically relevant threshold changes in anchor questionnaires. 3.3: Furthermore, after expansion of the patient group to 99 participants, content validity, consistency, reliability was confirmed, not only in the “pure” PDS group but also in PDS patients with overlapping non-predominant EPS.

Chapter 4: The measurement of intragastric pressure by means of high-resolution manometry during nutrient intake has recently been proposed as a potential minimally invasive alternative to assess GA. 4.1: IGP measurements showed that FD patients have lower nutrient tolerance and a smaller IGP drop during the intragastric infusion of the liquid meal compared to controls. Forty-five per cent of FD patients tolerated a maximum nutrient volume below the 10th percentile observed in controls. A moderate but significant correlation was seen between the nadir IGP and the amount of ingested meal, suggesting that subjects with more severe decreased nutrient tolerance are more likely to have a smaller drop in IGP during nutrient drink infusion, and by extension, poor gastric accommodation. Higher scores of pain, nausea and intestinal cramps during the intragastric infusion of the nutrient drink were also associated with a higher nadir IGP, indicating that a reduced drop in IGP is associated with higher postprandial dyspeptic symptom load. 4.2: Assessment of intragastric volume distribution by means of scintigraphy images during nutrient drink infusion, showed a clear relationship to the filling of the proximal stomach and a drop in IGP in the proximal stomach with satiation scores, rather than the filling and the IGP drop of the distal stomach. This indicates the importance of the proximal stomach as a reservoir and as determinant of satiation during ingestion of a meal. Overall, these data indicate that intragastric pressure measurement during a nutrient drink test has the potential to become a clinically relevant assessment of (patho)physiological factors and symptom generation.

Chapter 5: The effect of novel motility-modifying agents on gastric motility and sensitivity were studied by means of gastric barostat and IGP measurements. 5.1: Sildenafil citrate is a potent specific

PDE5 inhibitor which enhances NO diffusion in smooth muscles leading to muscle relaxation. Previous results with the barostat showed that sildenafil may enhance GA. On the other hand, its use in the treatment of erectile dysfunction is associated with dyspeptic symptoms as the most frequent adverse event. IGP measurement after an acute intake of sildenafil (PO, 50 mg), showed increased intragastric pressure, decreased nutrient tolerance, decreased gastric emptying rate and increased epigastric symptoms in healthy subjects. All are potentially relevant to dyspeptic symptom generation after use of this drug. 5.2: Prucalopride, a selective serotonin (5-HT4) agonist, is indicated for the treatment of chronic constipation, but was also shown to improve gastric emptying. Barostat measurements after an acute intake of prucalopride (PO, 2 mg), showed no significant effect on gastric accommodation but a tendency to increase gastric sensitivity. IGP measurements showed increased antral contractile activity associated to abdominal cramps. No effect was observed on IGP drop or nutrient tolerance. During the barostat study only, a large proportion of the subjects terminated earlier the study due to bothersome occurrence of nausea and the urge to vomit. This might be attributable to prucalopride-induced increased sensitivity to gastric distention and antral contractions alongside the intragastric barostat balloon. 5.3: Mirtazapine is a tricyclic antidepressant that has shown to improve symptoms, nutrient tolerance and body weight in FD patients with weight loss. After 3 weeks of treatment (PO, 15 mg) in healthy volunteers, IGP measurements showed a decrease of IGP drop. However, no decreased nutrient tolerance was observed, possibly because of central effects of the drug. During the barostat test, only a tendency to decreased gastric sensitivity was observed. The findings suggest that mirtazapine acts mainly centrally in improving FD symptoms and nutrient tolerance.

Chapter 6: The efficacy of prucalopride was studied in patients with idiopathic gastroparesis after a double-blind, randomized, placebo-controlled cross-over study. After 4 weeks of treatment prucalopride significantly enhanced gastric half emptying time compared to placebo and to baseline and also significantly improved upper abdominal symptoms such as nausea, fullness/satiety, bloating and reflux and quality of life compared to placebo.

Sammenvatting

Functionele dyspepsie (FD) is een van de meest voorkomende functionele gastro-intestinale aandoeningen en wordt gedefinieerd als “de aanwezigheid van maagklachten in afwezigheid van organische of metabole ziekte die de klachten kan verklaren”. De Rome consensus heeft voorgesteld om FD onder te verdelen in twee groepen met als doel het diagnosticeren en de behandeling van deze patiënten te optimaliseren. De eerste en grootste groep patiënten lijden aan het postprandiaal distress syndroom (PDS), dat gekarakteriseerd wordt door maaltijd gerelateerde klachten zoals vroege verzadiging tijdens maaltijd inname en extreem volheidsgevoel na de maaltijd. De tweede groep bevat patiënten die lijden aan het epigastrisch pijn syndroom (EPS), dat gekarakteriseerd wordt door maagpijn of een branderige gevoel in de maagstreek. In de klinische praktijk bestaat echter een overlap gemerkt tussen deze twee entiteiten van ongeveer 50%, wat het nut van de onderverdeling fel beperkt. De huidige therapeutische benadering van FD is gebaseerd op de vermindering van maagklachten door dieet-aanpassing en medicatie zoals zuurremmers en prokinetica. Nochtans is de doeltreffendheid van de huidige therapieën beperkt en wordt de ontwikkeling van nieuwe middelen belemmerd. De oorzaak hiervan is enerzijds de moeilijke selectie van patiënten voor klinische studies (door de overlap van de subgroepen en de heterogene pathofysiologie), het gebruik van niet-optimale eindpunten in FD studies in de laatste decennia, en het ontbreken van gevalideerde eindpunten voor klinische studies in FD. Het gegeven dat FD een heterogene en multifactoriële stoornis waarbij de pathofysiologie varieert, is een sterk beperkende factor, vooral indien deze pathophysiologie moeilijk kan onderzocht worden in de kliniek of in het kader van een behandelingsstudie. Een gestoorde maagrelaxatie bij de maaltijd of, anders gezegd, gestoorde gastrische accommodatie (GA) is een van de belangrijkste pathofysiologische mechanismen voor het ontstaan van klachten bij FD. De standaard methode om GA te meten is de maagbarostat, maar deze procedure is invasief, wordt door de patiënten als belastend ervaren en de barostat ballon die in de maag geplaatst wordt, interfereert ook met de fysiologische maagfuncties. Er is dus nood aan een nieuwe en gemakkelijkere techniek om GA in patiënten te kunnen meten.

Het doel van dit thesis onderzoek was om meer inzicht te verkrijgen in het symptoompatroon, de onderverdeling, de pathofysiologie en klinische eindpunten voor FD, om de diagnose en behandeling van functionele dyspepsie te verbeteren.

In hoofdstuk 2 voerden wij een systematische en gedetailleerde analyse uit van de klachten en kenmerken van patiënten met als doel de overlap tussen de verschillende subgroepen, PDS en EPS, te kunnen verminderen. Wij stelden vast op basis van vragenlijsten en metingen van symptomen na een standaard maaltijd, dat het grootse deel van de overlappende groep tussen EPS en PDS maaltijd gerelateerde klachten ervaart. Er werd geen relatie gevonden tussen de verschillende pathofysiologische mechanismen en de onderverdeling in EPS, PDS en de overlappende subgroep. Wij vonden enkel een opvallende gelijkenis in pathofysiologie tussen PDS en de overlappende subgroep. Op basis van deze observaties stellen wij voor om deze patiënten te identificeren en bij de PDS subgroep onder te brengen. De nadien resterende overlappende groep vertoont klachten zonder een duidelijke relatie met de maaltijd. De EPS subgroep werd duidelijk als een aparte groep geïdentificeerd bij alle studies en dit geeft aan dat de onderliggende pathofysiologie hier verschilt van die van de PDS en overlappende subgroep.

In hoofdstuk 3 beschrijven wij de ontwikkeling en validatie van een nieuwe vragenlijst die bedoeld is om de efficiëntie van behandelingen tijdens klinische studies in FD te meten. Deze vragenlijst werd

ontwikkeld voor PDS patiënten, omdat zij de grootste subgroep zijn en omdat er opportuniteiten zijn voor de ontwikkeling van nieuwe pro-kinetica voor deze subgroep. Om deze vragenlijst uit te werken werden eerst patiënten in interactieve sessies ondervraagd om de belangrijkste klachten in deze groep in kaart te brengen. Op basis van deze sessies werden vragen geformuleerd en de begrijpbaarheid en relevantie van deze vragen werd nagegaan in een bijkomende patiëntengroep. Na selectie van de beste bewoordingen werd een finale vragenlijst verkregen, in dagboekvorm, die de Leuvense postrandiale distress schaal (LPDS) werd genoemd. Uiteindelijk werden de validiteit, betrouwbaarheid en gevoeligheid van de vragenlijst nagegaan in 60 patiënten behandeld met placebo of itopride (een prokineticum) in een dubbel-blinde gecontroleerde studie. De LPDS vragenlijst werd door de patiënten dagelijks ingevuld gedurende een screeningsperiode van 2 weken gevolgd door een behandelingsperiode van 8 weken. Daarnaast vulden patiënten ook enkele referentie vragenlijsten, die standaard gebruikt worden in klinische studies, in. De validiteit van de LPDS werd aangetoond door de resultanten in de dagboekjes te vergelijken met de gegevens van de referentie vragenlijsten. Deze studie toonde aan dat de LPDS vragenlijst geschikt en accuraat is om veranderingen in de ernst van klachten aan te tonen. In een bijkomende analyse op een grotere patiëntengroep toonden wij aan dat de LPDS vragenlijst niet alleen accuraat is in PDS patiënten maar ook in PDS patiënten met overlappende EPS-klachten hadden. Door deze studie beschikken we nu over een gevalideerde vragenlijst met een verbeterde specificiteit en efficiëntie, die inmiddels ondersteund werd door het Europese Medisch Agentschap (EMA).

In hoofdstuk 4 en 5 werd een nieuwe techniek om gastrische accommodatie te meten gevalideerd, met name de intragastrische druk (IGD) meting met intragastrische toediening van een vloeibare maaltijd. Hierbij wordt een hoge resolutie manometrie sonde in de maag geplaatst en worden drukken voor en na toediening van een vloeibare maaltijd gemeten. Wanneer de vloeibare maaltijd wordt toegediend ontstaat een relaxatie van de proximale maag die door de manometrie sonde gemeten wordt als een daling van intragastrische druk die dus mogelijk de maagaccommodatie reflecteert. Om deze techniek te kunnen valideren en zijn bruikbaarheid te bepalen, onderzochten we eerst of via IGD een onderscheid kan gevonden worden tussen FD-patiënten en gezonde vrijwilligers. Vervolgens werd onderzocht of IGD metingen in gezonde vrijwilligers kunnen gebruikt worden om effecten fan farmacologische middelen op maagaccommodatie in te schatten. Bij gebruik van de IGD-metingen zagen wij inderdaad significante verschillen in intragastrisch drukverloop tussen FD patiënten en vrijwilligers. Patiënten vertoonden een verminderde daling van de intragastrische druk gedurende een vloeibare maaltijd, en dit was geassocieerd met vroegtijdige verzadiging en het ontstaan van maagklachten. In een simultane scintigrafie studie konden wij aantonen dat de verminderde IGD daling bij FD patiënten geassocieerd is met her-distributie van de maaltijd naar de distale maag, wat bevestigt dat dit een marker is voor gestoorde accommodatie. De studies met farmacologische middelen hebben aangetoond dat de IGD meting toelaat effecten van farmaca te detecteren bij gezonde vrijwilligers, en vertoonden een betere concordantie met nutriënt volume tolerantie dan de maagbarostat. Bovendien veroorzaakte de maagbarostat ook meer symptomen bij gezonde vrijwilligers. Deze data tonen dat ook aan dat de intragastrische druk meting het potentieel heeft om accuraat accommodatie te meten, wat relevante evaluaties in een klinische setting voor FD patiënten en voor farmacologische ontwikkeling toelaat.

In Hoofdstuk 6 bespreken we ook het gebruik van bepaalde middelen als mogelijk alternatief voor de behandeling van FD. Prucalopride werd eerst getest in gezonde vrijwilligers en leidde tot een

prokinetisch effect evenals een duidelijk toename van antrale contracties zonder de maag accommodatie negatief te beïnvloeden. Gedurende een behandeling met prucalopride gedurende 4 weken in een dubbelblinde placebo gecontroleerde cross-over studie in FD-patiënten met vertraagde maaglediging, trad een significante verbetering op van klachten, levenskwaliteit en maagontlediging in vergelijking met placebo. Deze observatie identificeert prucalopride als therapeutische optie voor de behandeling van FD met vertraagde maagleding.

Alhoewel FD een complex en heterogeen klinisch concept blijft, zetten wij in deze thesis een aantal stappen naar diagnostische en therapeutische verbetering. Wij bevestigden dat de maaltijd een belangrijke factor is bij het uitlokken van symptomen, niet alleen bij PDS maar ook bij de overlap groep. Een belangrijk pathofysiologisch mechanisme, de maagaccommodatie, kan gemakkelijker en accuraat geanalyseerd worden met behulp van intragastrische drukmeting. Prokinetica zijn vermoedelijk de anagewezen behandeling voor dergelijke patiënten, en wij ontwikkelden het LPDS dagboek als een gevalideerd eindmunt voor farmacologische klinische studies. Tenslotte toonden wij de efficiëntie aan van het prokineticum prucalopride in een piloot studie bij FD patiënten met vertraagde maagontlediging.

Curriculum Vitae

Maria Florencia Carbone was born January 13, 1989 in Santa Fe, Argentina. In 2003 she moved to Belgium and after completing her studies in "Mathematics Science 8 hours" at Sint-Jan Berchmanscollege in Genk, she started Biomedical Science at the Medicine Faculty of the University of Leuven (KULeuven) where she obtained the title of Master in Biomedical Science in June 2012.
In August 2012 she started a PhD at the Translational Research Center for Gastrointestinal Disorders (TARGID) of the Department of Clinical and Experimental Medicine at the KULeuven under supervision of Professor Dr. J. Tack. Her scientific research has contributed to several abstracts and peer-reviewed publications, and has led to the current PhD thesis.

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Awaiting publications

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Carbone F, Vanuytsel T, Tack J. The effect of prucalopride in gastric sensorimotor function and satiation in healthy volunteers. In preparation.

Carbone F, Vanuytsel T, Tack J. The effect of mirtazapine on gastric accommodation, gastric sensitivity to distention and nutrient tolerance in healthy subjects. In preparation.

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